ABSTRACT

Objective: We aimed to define the influence of different hypertension models on lipid peroxidation markers [conjugated dienes (CD) and thiobarbituric acid-reactive substances (TBARS)], antioxidant protection [paraoxonase-1 (PON1) activity] and visual evoked potential (VEP) changes in rats.

Methods: The study was designed as four different hypertension models. Rats (n=84) were divided equally into six groups: Control group (C), Sham operated (Sham), Two kidney-one clip (2K-1C), One kidney-one clip (1K-1C), Deoxycorticosterone acetate (DOCA) and N-omega-nitro-L-arginine-methyl ester (L-NAME). Brain TBARS, serum lipids (total and lipoprotein bound cholesterols and triglycerides) CD and TBARS levels and PON1 activity were assayed. Comparisons were performed using ANOVA or Wilcoxon/Kruskal-Wallis tests. Pearson correlation and linear regression analysis were used to evaluate associations of independent predictors with hypertension.

Results: Mean arterial pressure, brain and serum lipid peroxidation markers, VEP latencies were significantly higher in four hypertensive groups compared with control and sham groups (p<0.05). Compared with controls, PON1 activity was decreased in DOCA, 1K1C and L-NAME groups (p<0.05). Serum PON1 activity was negatively correlated with lipid peroxidation markers and VEPs. In terms of VEP’s records linear regression analysis showed that changes in N2 (β=1.51±0.34; p<0.001), P1 (β=-1.71±0.28; p<0.001), P3 (β=-0.54±0.14; p<0.001), serum TBARS levels (β=0.94±0.24; p<0.001) and PON1 activity (β=0.05±0.02; p<0.01) were independently associated with elevated blood pressure.

Conclusion: Lipid peroxidation measured in serum and brain was associated with increased electrophysiological alterations recorded as VEPs. This study might suggest that serum PON1 activity may be protective against brain and serum lipid peroxidation as well as electrophysiological alterations in the brain in different hypertension models. (Anadolu Kardiyol Derg 2014; 14: 498-504)

Key words: hypertension, lipids, oxidative stress, paraoxonase, visual evoked potential, regression analysis

Introduction

Hypertension affects approximately 25% of the adult population worldwide, 60% increase is predicted by 2025 (1). Hypertension is considered to be a multifactorial disease in which genetic and environmental factors, humoral and neural systems play a role (2). Although the exact mechanisms are unknown, accumulating evidence suggests that the tissue damage observed in hypertension is caused by increased free radicals leading to lipid peroxidation (3).

It is well known that free radicals generate a peroxidation cascade producing lipid peroxidation, protein oxidation, DNA damage and cell death, and contribute to the occurrence of pathologic conditions (4). Lipid peroxidation is believed to be involved in several disease states, such as diabetes and neurodegenerative diseases as well as the ageing process (5). Several lines of evidence suggest that hypertension is associated with enhanced oxidative stress (6). Since the brain is particularly sensitive to the effects of oxidative stress due to its very high rate of oxygen consumption and the non-regenerative nature of its neurons, it is likely that the most serious effects of lipid peroxidation may be exerted in the nervous system (7).

Although the mechanism underlying the increased oxidative stress in hypertension is not completely understood, it has been generally accepted that an increase in vascular reactive oxygen species (ROS) contributes to the genesis of hypertension. It is highly likely that an increase in blood pressure and/or other factors elevates ROS in the arterial wall by reducing the activity of antioxidant enzymes and/or enhancing the activity of the enzyme systems that are capable of producing ROS (6). Paraoxonase-1 (PON1) is an antioxidant esterase synthesized in the liver that circulates in the plasma, bound exclusively to HDL (8).
Paraoxonase stimulates hydrolysis of lipid peroxides (9). Although a small number of studies have attempted to show that reduction in serum PON1 activity, might result in increased oxidative stress and lipid peroxidation products in hypertension (6), we did not encounter any study on animal hypertension models investigating the relationship between serum PON1 activity and lipid peroxidation.

Previous data revealed that hypertension is associated with significant prolongation in the latencies of visual evoked potential (VEP) components which represent a sensitive method for detecting the involvement of optic pathways responsible for the earliest alterations in the visual system (10). VEPs are known to be sensitive and highly reliable indicators of the visual system changes and consist of several components arising from retina, optic pathway, subcortex and cortex (11). It is an electrophysiological method for detecting the earliest alterations in the visual system and can be used in experimental studies since the fact that VEP recordings from rats are good models for studying human visual system (10, 12). It would be quite difficult to prospectively examine factors effecting VEP recordings in hypertensive humans since such changes may take many years to manifest and even more difficult to obtain brain samples for lipid peroxide comparisons. One approach is to develop an animal model with a sufficiently short life span in which some conditions of hypertension can be stimulated. We chose the rat as a suitable animal in which to examine the effects of lipid peroxidation and VEP changes on hypertension because of extensive knowledge about mechanisms of systemic hypertension that have been developed in this model, many of which have also been demonstrated in humans (12). As far as we are aware, the relationship among serum and brain lipid peroxidation markers and VEP recordings in hypertension were not evaluated in animals or human before. For this reason, the aim of the present study was to be able to investigate possible relationships between lipid peroxidation markers measured both in central (brain) and peripheral (serum) samples with antioxidant enzyme activities in four different hypertension models and discuss their influence on brain electrophysiological alterations defined as VEP changes.

Methods

Study design

The procedures were reviewed and approved by the Akdeniz University Experimental Animals Local Ethical Committee (AKHADYEK). Animal care was in accordance with the 15 October 1978, UNESCO “The Universal Declaration of Animal Rights”, 18 March 1986, The European Council, ETS 123 “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose” and ILAC/NRC Guidelines for the care and Use of Laboratory Animals and all procedures were reviewed and approved for Animal Research Ethics. Male Wistar rats (n=84) obtained from Akdeniz University Experimental Animals Unit, aged 3 months (200-250 g) were used in this study. Animals were housed in groups of 4-5 rats in stainless steel cages at standard conditions (24±2°C) with 12-hour light-dark cycles.

Drugs and treatment schedule

The animals were randomly divided into six groups consist of 14 animals in each: Control group (C), Sham operated (Sham), two kidney-one clip (2K-1C), one kidney-one clip (1K-1C), deoxy-corticosterone acetate (DOCA) and N-omega-nitro-L-arginine-methyl ester (L-NAME). The doses and different hypertension models were selected on the basis of literature (13). The DOCA hypertension first had a right nephrectomy and than rats were treated twice weekly with DOCA suspended in corn oil, which was administered subcutaneously (15 mg/kg) and 1% NaCl added to their tap water for drinking. Hypertension was induced by placing a silver clip (0.2 mm ID) across the left renal artery, exposed under anesthesia with the right kidney left untouched in 2K-1C model. In 1K-1C hypertension model, after being anesthetized, the right kidney was removed and a silver clip with a 0.2 mm gap was placed on the left artery. In L-NAME hypertension model, L-NAME was injected intraperitoneally at a dose of 50 mg/kg/day (75 days) to induce increase in blood pressure via a nonselective inhibition of nitric oxide synthase (NOS).

Rats were maintained on normal rat chow and were allowed to drink tap water. Daily food and water consumption of every cage and weekly weight of individual rats were recorded during the feeding period. At the end of the 10-weeks experimental period, rats were deprived of food for 24-hours and then prepared for experimental procedure under ether anesthesia.

Mean blood pressure measurement

Mean blood pressure was monitored at weekly intervals by the tail cuff method (14) using Data Acquisition System (MP100A-CE: MAY-BPHR200). Each value was the average of two consecutive readings.

VEP recordings

Visual evoked potentials were recorded by a single investigator in a blinded manner. with stainless steel subdermal electrodes (Nihon Kohden NE 223 S, Nihon Kohden Corporation, Tokyo 161, Japan), which were placed 0.5 cm in front of (reference electrode) and behind bregma (active electrode) as previously described (15). A ground electrode was placed on the tails of animals. After 5 min of dark adaptation, a photic stimulator (Nova-Strobe AB, Biopac System Inc., Santa Barbara, CA 93117, USA) at the lowest intensity setting was used to provide the flash stimulus at a distance of 15 cm, which allowed lighting of the entire pupilla from the temporal visual field. Repetition rate of the flash stimulus was 1 Hz and the flash energy was 0.1 J. VEP recordings from both right and left eyes were obtained and throughout the experiments the eye not under investigation was occluded by appropriate black carbon paper and cotton. Meanwhile, body temperature was maintained between 37.5°C and 38°C by a heating pad. The averaging of 100 responses was
accomplished with the averager of Biopac MP100 data acquisition equipment (Biopac System Inc., Santa Barbara, CA 93117, USA). Analysis time was 300 ms. Frequency bandwidth of the amplifier was 1Hz-100Hz. The gain was selected as 20-50 μV/div. At least two averages were obtained to ensure response reproducibility. Peak latencies of the components were measured from the stimulus artifact to the peak in ms. Amplitudes were measured as the voltage between successive peaks. The animals were carefully handled to avoid variation in the measurement. The VEP recordings were evaluated randomly by another neurologist blinded to the recording procedure.

Sample collection
Animals were anesthetized with urethane (1 g/kg) and their abdomens were opened by a midline incision. Blood samples were taken and animals were sacrificed by cardiac puncture. The brain tissues were removed. Brain tissue homogenates were used for TBARS analysis whereas; blood samples were used for the analysis of serum TBARS, conjugated dienes and lipid levels, and paraoxonase-1 activity.

Lipid assays
Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured enzymatically using commercial kits (Abbott Aeroset system, Laboratories, Abbott Park, Illinois). The low density lipoprotein cholesterol (LDL-C) fraction was calculated indirectly using the Friedewald equation (16). The factor (triglyceride)/5 was used to estimate very low density lipoprotein cholesterol (VLDL-C) concentration (16).

Paraoxonase-1 (PON1) activity assay
Serum PON1 activity was assayed spectrophotometrically as described previously (17). Briefly, the assay mixture consisted of 1500 μL of 6 mmol/L paraoxon substrate solution in 0.1 mol/L Tris-HCl buffer, pH 8.0, containing 1 mmol/L calcium chloride (CaCl2) and 60 μL of fresh serum specimen. The absorbance was monitored photometrically at 405 nm and at 37°C at 1 minute intervals on a spectrophotometer (photometer 4010, Boehringer Mannheim, GmbH, Germany). One unit of PON1 activity was defined as 1 μmol of p-nitrophenol formed per minute and the activity was expressed as U/L of serum.

Conjugated diene (CD) assay
Conjugated diene levels were measured by the method of Recknagel and Glende (18). Lipids were extracted with 2:1 (v/v) chloroform-methanol; the extract was evaporated to dryness under a stream of nitrogen and then redissolved in cyclohexane. The cyclohexane solution was assayed at 234 nm. The results were expressed as μmol/L using εmax=25.200 M⁻¹ cm⁻¹.

Thiobarbituric acid reactive-substances (TBARS) assay
Thiobarbituric acid reactive-substances levels were measured by a fluorimetric method described by Gümüşlü et al. (19) with minimal modifications described by Wasowicz et al. (20) using 1,1,3,3-tetramethoxypropane as a standard and results were given as nmol/mg protein for tissue samples and μmo MDA/L for serum, with a spectrofluorometer (Shimadzu RF-500, Kyoto Japan) using wavelengths of 525 nm for excitation and 547 nm for emission.

Statistical analysis
To reduce possible bias, we performed a power analysis on the basis of available data and designed the study to detect statistically significant difference with a power of 0.85 and α=0.05. Statistical analyses were performed using SPSS version 16.0 (SPSS Inc. Chicago, IL). Normality tests were applied using the Kolmogorov-Smirnov criterion. Results are expressed as mean±SEM. Comparisons among groups were performed using ANOVA, with the Tukey/HSD test for multiple comparisons. In case of non-normal distribution, nonparametric tests (Wilcoxon/Kruskal-Wallis) were applied to compare among the groups. Linear regression analysis was used with forward and backward modeling to evaluate association of independent predictors that were significantly associated with hypertension. Pearson test was used for correlation analysis. Statistical significance was accepted for p<0.05.

Results
At the end of the study, body weight did not significantly differ between the groups (p>0.05). Water intake increased in parallel with food intake in 2K1C and 1K1C hypertensive rats (p<0.05) compared with sham group. On the other hand, compared with C group, treatment with DOCA or L-NAME elevated water intake without a significant change in food consumptions (p>0.001, Table 1).

Mean arterial blood pressure was significantly elevated in hypertensive rats (DOCA, 2K-1C, 1K-1C, L-NAME, p<0.0001) when compared with their age matched control and sham-operated counterparts. There was no significant difference in blood pressure among the hypertensive groups (Table 1).

Serum levels of TC, HDL-C, LDL-C, VLDL-C and TG were not different among groups (p>0.05, Table 2).

Lipid peroxidation was measured in serum and brain samples. Our data indicated that hypertension condition caused an increase in lipid peroxidation of four hypertension groups (p<0.001), compared with control and sham groups. There was also a significant increase in the brain TBARS level of DOCA rats as compared to L-NAME group. When we compared the serum CD and TBARS levels, there was no significant difference among the hypertensive groups (p>0.05, Table 3). Serum PON1 activity was found to be decreased in hypertensive groups (p<0.05, Table 3); whereas decrease was distributed in two different areas (Fig. 1). In DOCA and 1K-1C groups the inactivation was observed in both areas while 2K1C and L-NAME groups had decreased PON1 activity in higher PON1 activity area with respect to controls (p<0.05, Fig. 1).

In terms of VEPs records, the mean latencies of P1, N1, P2, N2 and P3 components were detected to be significantly prolonged in all hypertensive groups as compared to control and sham groups (p<0.001). However, the mean latencies of all VEPs
components in the L-NAME group were longer than those in other hypertensive groups (Table 4).

Serum PON1 activity was negatively correlated with serum CD \( (r = -0.570, p < 0.001) \) and brain TBARS levels \( (r = 0.834, p < 0.001) \) and brain TBARS levels \( (r = 0.877, p < 0.001) \) and P1 \( (r = 0.557, p < 0.001) \), N1 \( (r = 0.699, p < 0.001) \), N2 \( (r = 0.745, p < 0.001) \) and P3 \( (r = 0.785, p < 0.001) \). Serum CD levels were positively correlated with brain TBARS levels \( (r = 0.847, p < 0.001) \), and P1 \( (r = 0.489, p < 0.001) \), N1 \( (r = 0.542, p < 0.001) \), P2 \( (r = 0.701, p < 0.001) \), N2 \( (r = 0.728, p < 0.001) \) and P3 \( (r = 0.600, p < 0.001) \).
Figure 1. PON1 activity frequency in experiment groups
1K-1C - one kidney-one clip; 2K-1C - two kidney-one clip; DOCA - deoxycorticosterone acetate; L-NAME - larginine-methyl ester; PON1 - paraoxonase-1; Sham - sham operated

Linear regression analysis showed that changes in N2 (B=1.51±0.34; p<0.001), P1 (B=-1.71±0.28; p<0.001), P3 (B=0.54±0.14; p<0.001), serum TBARS levels (B=0.94±0.24; p<0.001) and PON1 activity (B=0.05±0.02; p<0.01) were independently associated with elevated blood pressure.

Discussion

In the present study, we report the association of serum lipid peroxidation markers and PON1 activity with VEP changes in hypertension. Regression analysis also revealed that PON1 activity and serum TBARS were associated with hypertension. These results suggest that elevated blood pressure may be related with the “lipid peroxidation mediated” electrophysiological changes in the brain. Thus, whereas previous studies demonstrated that hypertension is associated with well known functional and structural vascular alterations that may result from the interplay of several mechanisms (21), the current study is the first to report in different hypertension models that decreased PON1 activity might be a risk factor for reduced physiological activity of the brain, in particular, in patients with chronic hypertension.

The hypertension duration was 10 weeks in all hypertension groups. Therefore, the changes in different parameters among groups were not related with blood pressure or the duration of hypertension. Increased water turnover was described in some of the earliest reports of experimental DOCA hypertension (22). Our study confirms the report of Allan et al. (23) who reported that DOCA treated animals consumed more water, on the other hand L-NAME group also had increased water consumption without a significant change in food consumptions. This increment in water consumption might be attributed to increased salt consumption (23).

Lipid peroxidation, arising from the reaction of free radicals with lipids, has been linked with altered membrane structure and enzyme inactivation. Its end products are measured as TBARS, lipid hydroperoxides and conjugated dienes (24). In our study, all hypertensive groups had increased serum CD and TBARS indicating increased production of toxic aldehydes in hypertensive rats as previously reported (24). This enhancement of lipid peroxidation products might be due to increased tissue damage, free radical production and decreased hydrolysis of lipid peroxides (24).

It is known that hypercholesterolemia is associated with increased levels of oxygen free radicals and lipid peroxidation which leads to tissue damage (25). In clinical studies, abnormalities of TC and lipoproteins were reported in essential hypertension (26). However we observed that hypertension was not accompanied with altered serum lipid levels in four different hypertension models. A possible explanation for this is that hypertension induced lipid peroxidation might result from insufficient elimination of these toxic products rather than the serum lipid content as a generating factor.

In accordance with the clinical findings that hypertensive patients have decreased PON1 activities compared to normotensives (27), we similarly found PON1 activities to be significantly decreased in all hypertensive groups. We are also in agreement with Uzun et al. (6) who demonstrated that due to an increase of blood pressures, oxLDL and MDA increased while PON1 decreases. In other words, oxidative stress increases, while antioxidant activity decreases as the blood pressure rises (6). This decrement may be due to enhanced lipid peroxidation end-products suggesting that these peroxides could be inactivating PON1 via feedback mechanism or may be because of decreased scavenger or hydrolysing activity of PON1.

We found the mean latencies of P1, N1, P2, N2 and P3 components significantly prolonged in all hypertensive groups as compared to control and sham groups. These VEP changes may be due to the favor of increased lipid peroxidation as expected. Because of that all of the groups had increased serum CD and TBARS levels having a positive correlation with VEP latencies. Therefore, it could be concluded that lipid peroxidation might have a role in the prolongation of all VEP components. It is well known that free radicals cause damage in biological macromolecules and lead to lose their cellular functions. In particular, oxidation of lipids found abundantly in cell membranes leads to altered membrane structure and changes in neuronal functions (28). This view is supported by our results indicating that lipid peroxidation increases electrophysiological alterations recorded via VEPs. Increased ROS concentrations play an important role in the vascular dysfunction found both in clinical and experimental hypertension. High levels of superoxide react with NO in the aortic endothelium, thus reducing its bioavailability and leading to endothelial dysfunction and impaired vascular relaxation (21). The mean latencies of all VEPs components in the L-NAME group were found to be longer than those in other hypertensive groups, indicating that NO may play an important role in visual system.

Study limitations

The lack of data at the different time points of hypertension. Therefore, future studies designed to quantify the relative input...
of each mechanism will be needed for a more comprehensive understanding.

**Conclusion**

Decreased PON1 activity and increased serum TBARS were found to be risk factors in hypertension. An inverse relationship was observed between PON1 activity and lipid peroxide content in brain and serum samples of different hypertensive models. Decreased PON1 activity and increased lipid peroxidation were also associated with electrophysiological alterations recorded via VEPs. This study might suggest that serum PON1 activity may be protective against brain and serum lipid peroxidation as well as electrophysiological alterations in the brain in different hypertension models.

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