Short Reports

Oxidative stress in patients with essential hypertension

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ABSTRACT

Background. Free oxygen radicals react with membrane lipids to form lipid hydroperoxides, a destructive process known as lipid peroxidation. Lipid hydroperoxides decompose to form a variety of products including malondialdehyde, which is used as an indicator of the oxidative damage of cells and tissues. Endogenous antioxidant enzymes such as superoxide dismutase counteract the oxidative damage from oxidative stress.

There is increasing evidence that free radicals are involved in the pathogenesis of hypertension by altering endothelial function. We evaluated the oxidative stress and endogenous enzymatic antioxidant status in patients with essential hypertension before and 3 months after treatment with antihypertensives.

Methods. Fifty patients with essential hypertension attending the outpatient services of the Department of Medicine, Institute of Medical Sciences, Banaras Hindu University and 20 age- and sex-matched healthy controls were studied. The serum malondialdehyde and superoxide dismutase levels were measured in patients at the time of presentation and after 3 months of antihypertensive treatment. No antioxidants were given to the patients during the period of the study.

Results. The mean (SD) serum malondialdehyde level was found to be significantly higher (0.33 [0.07] mmol/L) in patients with hypertension compared with controls (0.21 [0.05] mmol/L; $p < 0.001$). This showed a significant decrease following antihypertensive therapy (0.23 [0.06] mmol/L; $p < 0.001$) compared with pre-treatment values. The serum superoxide dismutase activity was significantly lower in patients (6.93 [1.35] mg protein/ml of serum) compared with controls (20.12 [3.65] mg protein/ml serum; $p < 0.001$) at the time of presentation and, compared with the pre-treatment values, increased significantly after 3 months of treatment (10.66 [2.91] mg protein/ml of serum; $p < 0.001$).

Conclusion. Our study shows that essential hypertension is associated with increased oxidative stress and reduced antioxidant status. Adequate control of blood pressure with antihypertensive therapy decreases oxidative stress and improves the antioxidant status in these patients.

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INTRODUCTION

The term 'hypertension' denotes a systolic blood pressure (SBP) of ≥140 mmHg, diastolic blood pressure (DBP) of ≥90 mmHg or a patient taking antihypertensive medication. In most cases, hypertension is idiopathic and is referred to as essential or primary hypertension. Hypertension may lead to serious complications, which include heart failure, coronary artery disease, valvular heart disease, cardiac arrhythmia, cardiomyopathy and abnormal renal function. The pathogenesis of essential hypertension may include a number of factors such as heredity, increased fluid volume, renal sodium transport deficiency, increased sympathetic activity and increased vascular tone, involvement of the renin–angiotensin–aldosterone system, chronic stress, diminished activity of vasodepressor hormones (prostaglandins, atrial natriuretic factor), etc. In addition, factors such as obesity, physical inactivity, occupation, smoking, alcoholism, etc. may also contribute. In less than 5% of cases, hypertension is secondary to an identifiable cause such as renal, endocrine and neurological abnormalities, or congenital cardiovascular anatomical defects or iatrogenic causes.[1]

Oxidation occurs when high levels of oxygen react with exposed surfaces during respiration. It happens every time we take in a breath of air. Free radicals are byproducts of this process. Free radicals are unstable molecules that react with normal, healthy body cells and damage them. This repetitive damage leads to degenerative diseases such as cancer, ischaemic heart disease, diabetes, arthritis, cataract and ageing, among others.

Free radicals are produced in the body both deliberately and as a byproduct in daily metabolic processes. Oxygen is required by various systems for the release of energy and to detoxify xenobiotics. The products of partial reduction of oxygen are referred to as 'reactive oxygen species' which are highly reactive.[2] Various antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase control the accumulation of free radicals. A serious imbalance between the production of free radicals and protection by antioxidants leads to oxidative stress. If the defence mechanism of the body fails to combat free radicals, they can injure tissues and their DNA, as well as the protein or lipid components of cells, leading to cell damage and cell death.[3] Endogenous antioxidants such as SOD and glutathione counteract oxidative damage. An imbalance between the production of free radicals and antioxidants leads to endothelial dysfunction caused by the free radicals. As an antioxidant, SOD catalyses the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen, protecting the cells against the potential toxicity of reactive oxygen; hydrogen peroxide is further detoxified by catalase to water.

We studied the levels of malondialdehyde (MDA), a marker of lipid peroxidation and the status of SOD in hypertensive patients before and after 3 months of antihypertensive therapy.

METHODS

Fifty patients with essential hypertension attending the outpatient services of the Department of Medicine, University Hospital,

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Banaras Hindu University, Varanasi were included in the study. Hypertension was defined according to the criteria of the VI Joint National Committee.[1] Clinical assessment included a history and physical examination. Twenty healthy, normotensive non-smokers, matched for age and sex were included as controls. Patients were given antihypertensive treatment for 3 months with beta-blockers and diuretics but without any antioxidants. Informed consent was obtained from all the patients.

Measurement of blood pressure

Blood pressure was measured with a mercury sphygmomanometer, with the patient in the sitting position after 5 minutes of rest in a quiet environment, as recommended by the British Hypertension Society.[4] Three readings were obtained at 5-minute intervals and their average was taken for analysis.

Collection of blood samples

Blood samples were collected from the antecubital vein of the patients as well as controls in the morning before taking any meals. Lipid peroxidation and antioxidant enzyme levels were assayed from the serum.

Lipid peroxidation assay

The lipid perodixation assay in the serum was performed by the technique of Philpot[5] as modified by Buege and Aust.[6] The stock reagent contained 15% trichloroacetic acid (TCA), 0.375 g 2-thiobarbituric acid (TBA) and 0.25 N hydrochloric acid (HCl) per 100 ml solution. A fresh stock of TBA–TCA–HCl was prepared at the time of the assay, and 0.01% butylated hydroxytoluene was added to the stock reagent to abolish metal catalysed auto-oxidation of lipids.[7] The standard MDA solution was prepared from MDA bis(dimethyl) acetal solution obtained from Aldrich Chemicals, USA. Two ml of the stock reagent was added separately to 1 ml of the test, standard and blank samples and all the samples were heated for 20 minutes at 80 °C and then allowed to cool at room temperature. The pink pigment from all the samples was then extracted with 4 ml of *N*-butanol and the optical absorbance was recorded at 530 nm on a spectrophotometer (SICO, India).

Assay of SOD in serum

The SOD assay was based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol.[8] The assay was performed by taking 2.5 ml tris buffer (0.05 M) and 0.5 ml ethylene diamine tetra acetic acid (EDTA; 1 mmol) as the blank, 1.5 ml tris buffer, 0.5 ml EDTA (1 mmol) and 1 ml pyrogallol (0.2 mmol) as the control, and 1.5 ml tris buffer (0.05 M), 0.5 ml EDTA (1 mmol), 0.05 ml serum and 1 ml pyrogallol (0.2 mmol) as the test. The change in optical absorbance of the test sample per minute with reference to the blank sample was recorded at 420 nm on the spectrophotometer. The enzyme inhibition caused by the serum was calculated and the enzyme activity expressed in mg protein/ml of serum.

Statistical analysis was done using the Student *t* test to determine the difference between the means of the two groups.

RESULTS

The hypertension group included 31 men and 19 women and their mean (SD) age was 56 (9) years. There were 14 men and 6 women in the control group with a mean (SD) age of 48 (12) years. All the controls were normotensive and had slightly lower total cholesterol levels than the patients. Among the patients, 9 had total cholesterol levels >225 mg/dl, 9 had low-density lipoprotein levels >135 mg/dl, 4 had high-density lipoprotein levels <35 mg/dl and 2 had hypertriglyceridaemia (>185 mg/dl).

TABLE I. Mean (SD) blood pressure in patients with hypertension

Blood pressure	Hypertension			Mean
	Mild (*n*=22)	Moderate (*n*=23)	Severe (*n*=5)	
Systolic	152 (4.5)	162 (6.3)	180 (9.0)	169 (12.9)
Diastolic	98 (2.2)	100 (5.9)	104 (8.6)	102 (7.2)

The patients were grouped into those with mild, moderate and severe hypertension based on the JNC-VI criteria (Table I). Following treatment for 3 months with antihypertensives the mean (SD) SBP was 125 (7) mmHg and the mean (SD) DBP was 80 (6) mmHg.

Lipid peroxidation and superoxide dismutase assays

The mean (SD) baseline MDA level in patients with hypertension was 0.33 (0.07) mmol/L and was significantly higher than that of controls (0.21 [0.05] mmol/L; $p<0.001$). After 3 months of antihypertensive treatment, the MDA level decreased significantly to 0.23 (0.06) mmol/L compared with the pre-treatment level ($p<0.001$). The enzymatic activity of SOD, on the other hand, was significantly lower in patients (6.93 [1.35] mg protein/ml of serum) compared with controls (20.12 [3.65] mg protein/ml of serum; $p<0.001$). After 3 months of antihypertensive treatment, the SOD activity had increased significantly (10.66 [2.91] mg protein/ml of serum; $p<0.001$).

DISCUSSION

We examined the activity of the most important antioxidant enzyme, SOD and lipid peroxidation levels in patients with hypertension before and after 3 months of antihypertensive treatment. The baseline results indicated high levels of lipid peroxidation along with decreased activity of SOD compared with normotensive controls. Following 3 months of antihypertensive treatment, these values were comparable with those in the controls and had increased significantly in case of SOD from the pre-treatment baseline values. However, Redon *et al*.[4] observed no relationship between oxidative stress and blood pressure values. Possibly, measures of circulating reactive oxygen species do not adequately reflect oxidative stress in the vascular wall, since the enzymes that increase reactive oxygen species have different regulatory mechanisms in the endothelial cells than in peripheral mononuclear cells.[4]

MDA is the most abundant among the reactive aldehydes derived from lipid peroxidation. These aldehydes have been implicated as the causative agents in cytotoxic processes, and it is possible that aldehydes released from the cell membrane may diffuse, interact and induce oxidative modifications in other cells and in low-density lipid molecules, thereby increasing the risk of cardiovascular damage.[9]

These observations suggest that hypertensive patients are prone to oxidative damage, which is also evident from the improvement in the activity of SOD following control of blood pressure. Similar observations have been reported in the literature.[10,11] However, it is unclear whether the activity of antioxidant enzymes is the cause or the consequence of the increased oxidative stress. The fact that the low activity included several systems points to the reduction being more a consequence than a cause.[4,10]

Our study reveals that oxidative stress is increased in patients

with hypertension but its pathogenetic and clinical relevance remain to be elucidated.

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