ACTIVITY OF XANTHINE OXIDASE IN DIABETICS: ITS CORRELATION WITH AGING

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Summary

The adverse role of free radicals has been implicated in many disease conditions and also in aging. The endogenous antioxidant defenses comprising of enzymic and non-enzymic components monitor and neutralize the free radicals and thus prevent injury to proteins, lipids and DNA. The major sources of free radicals include the electron transport chain and the reactions catalysed by few oxidases. Xanthine oxidase (XO) is an enzyme which converts hypoxanthine/xanthine to uric acid in a reaction which liberates superoxide. High activity of XO has been observed in many pathological conditions as the XO-generated superoxide is cytotoxic. Diabetes is a disease which is associated with many pathophysiological complications arising due to oxidative stress. The aim of this study was to analyse the activity of XO in diabetics and to assess its correlation with the aging of diabetics. A significant increase in the activity of XO was observed in the diabetics and a marked increase was observed with aging.

Key words: aging, free radicals, diabetes, xanthine oxidase.

Introduction

Formation of reactive oxygen species (ROS) like superoxide, hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), singlet oxygen, ozone, lipid peroxides is associated with pathological conditions and aging in animals. Endogenous enzymic and non-enzymic anti-oxidant systems are crucial for the control of ROS mediated injury to macromolecules like proteins, lipids and nucleic acids. The prime sources of ROS include the leaky mitochondrial chain and enzymes like NADPH oxidase, cytochrome oxidase and xanthine oxidase (1). However, when the production of ROS overcomes the capacity of antioxidants, oxidative stress ensues (2).

Xanthine oxidase (XO) is a cytosolic enzyme which oxidizes a wide range of substrates encompassing purines and pyrimidines, many endogenous compounds and clinically used drugs. XO exists in its dehydrogenase form (XDH) under normal conditions. Under pathological conditions, XDH undergoes oxidation or proteolysis to get converted to XO (3). The primary role of XO is to act on hypoxanthine and xanthine and convert them to uric acid with the production of a superoxide ion. XO-derived superoxide reacts with nitric oxide to form peroxynitrite which is cytotoxic. This leads to oxidative and nitrosative injury to proteins, lipids and DNA in the vicinity of XO (4). High activity of XO has been reported in various pathological states like myocardial infarction, hepatic diseases, asthma (5, 6)

Diabetes is a devastating disease characterized by severe morbidity and mortality. The clinical management of diabetes is complex owing to the occurrence of severe vascular complications. The onset and progression of all the pathophysiological complications of diabetes involves oxidative stress. The commonly reported mechanisms for increased oxidative stress in diabetes are increased formation of advanced glycation end products, altered glutathione homeostasis, impaired superoxide dismutase and catalase and induced aldose reductase (7). The role of XO in diabetes has been less explored. This study hence aimed at investigating the alterations in XO activity in diabetes and analysing its correlation with the age of the diabetics.

Materials and methods

Study subjects

Eighty volunteers participated in this study. The subjects included 40 men and 40 women. Of these, 20 men and 20 women were diabetic and the remaining 40 subjects were non-diabetic controls. Both the diabetic and non-diabetic subjects were grouped in to two; one group were 30 - 50 years old with a median age of 41 years and the other group were aging above 50 with a median of 62 years. All the diabetic subjects were diagnosed of diabetes for minimum 5 yrs and maximum 8 years. All the diabetic volunteers were under treatment and had good control over their blood sugar and thus were not hyperglycemic. Informed consent was obtained from all the volunteers.

Assay of XO activity

Assay of XO activity was done by the method of Haidari et al. (8) with few modifications. The activity of XO was assayed by monitoring the production of uric acid from hypoxanthine. The reaction mixture consisted of 1.0 ml of hypoxanthine (20 μg/ml), 1.9 ml of phosphate buffer (0.5M, pH 7.5) and 0.1 ml of whole blood. After 30 min, the reaction was arrested by the addition of 0.5 ml HCl (0.6 M). The reaction mixture was centrifuged and the supernatant was harvested. To the supernatant, 0.6 ml of phosphotungstic acid and 0.6ml of sodium carbonate was added and the tubes were read at 640nm after 20 min.

Estimation of Lipid peroxidation

The extent of lipid peroxidation was determined as the concentration of thiobarbituric acid reactive substances (TBARS), according to the method of Ohkawa et al. (9). Briefly, 100 µL of whole blood or malondialdehyde (MDA) standards were pipetted into test tubes containing 1.5 mL of 20% (w/v) glacial acetic acid (pH 3.5), 200 µL of 8.1% (w/v) sodium dodecyl sulphate (SDS), 1.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) and 700 µL of distilled water. The test tubes were incubated at 95 °C for 60 minutes with a marble on top of each test tube. After incubation, the test tubes were cooled and then centrifuged at 3000 × g for 10 minutes. The amount of MDA formed was measured spectrophotometrically at 532 nm. 1,1,3,3-Tetraethoxypropane (TEP), a form of MDA, was used as standard in this assay. TBARS concentration was expressed as nmol of MDA per mg protein.

Estimation of protein

Protein was estimated by the method of Lowry et al. (10).

Statistical analysis

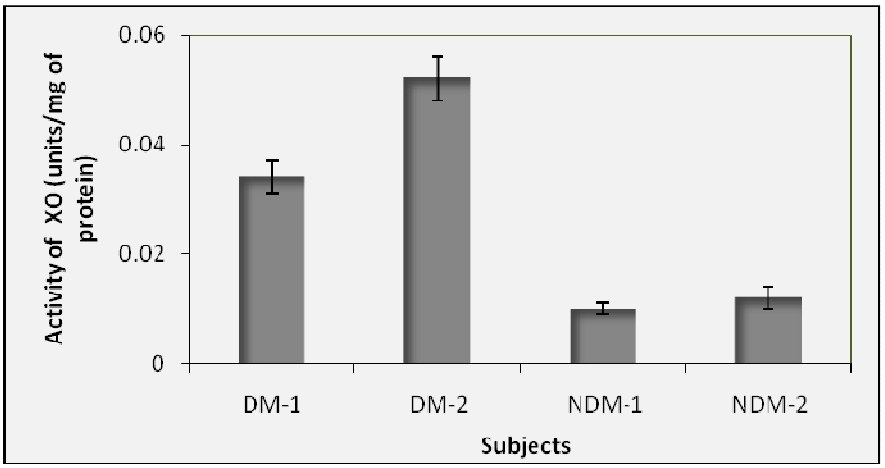
All values are reported as mean ± SEM. Statistical significance was assessed using Student's t-test.

Results and Discussion

In recent years, free radicals and other reactive intermediates produced in normal metabolic processes have been implicated in the pathogenic mechanism of a wide range of diseases, including inflammatory diseases, cancer, atherosclerosis, and liver injury. Metabolic free radical sources include XO, prostaglandins (PG), hydroperoxidase, NADPH dehydrogenase, mitochondrial electron transport, and peroxisomal enzymes such as amino acid oxidase and fatty acyl CoA oxidase (11). Xanthine oxidase - derived superoxide exerts its actions on various endogenous oxidant and antioxidant systems. Following the binding of the circulating XO to the endothelium, XO-derived superoxide can combine with endothelially derived NO which is produced by the endothelial NO synthase. The subsequent formation of peroxynitrite triggers various downstream pathways of cell injury which can lead to endothelial and tissue injury in various pathophysiological conditions (4). Though XO has been well investigated in clinical conditions like lung and liver injury, myocardial infarction (5), its role in diabetes and its pathophysiology has been less explored. Hence the aim of this study was to analyse the activity of XO in diabetics.

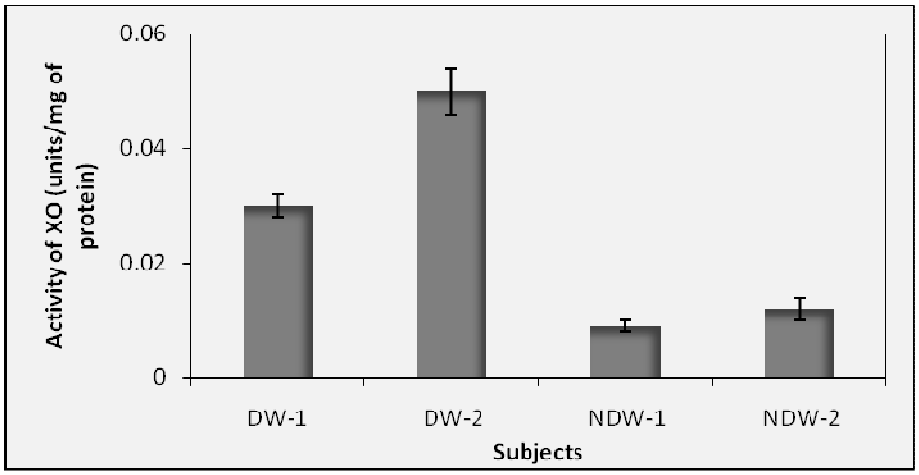
XO activity was analysed in male and female diabetic and nondiabetic subjects. The activity of XO was found to be high in all the diabetic subjects, irrespective of the sex. A significant increase ($p<0.001$) in the activity of XO was observed in diabetic men and women below 30 years of age when compared to non-diabetic controls. Similarly, the activity of XO in diabetic subjects aging between 30 and 50 was also found to be significantly high, with $p<0.0001$ in diabetic women and $p<0.005$ in diabetic men. The difference in activity of XO was not significant when sex-dependent XO activity was analysed (Figure 1 and 2).

Figure 1. Activity of Xanthine oxidase in diabetic men



DM- diabetic men, NDM – non-diabetic men, 1 – below 30 years of age; 2 – aged between 30-50 years

Figure 2. Activity of Xanthine oxidase in diabetic women



DW – diabetic women; NDW – non-diabetic women, 1 – below 30 years of age; 2 – aged between 30-50 years

The tissues that express the highest activity of XO are liver and intestine. The increase in XO activity in diabetes could be attributed to the release of XO from liver. The release of xanthine oxidase from liver has been observed not only in diabetes but also in other pathological states, such as hemorrhagic shock. However, the release is not due to non-specific membrane damage. Xanthine oxidase is shed by the liver into the plasma and is bound to vascular endothelial cells. It has been proved that arterial rings from diabetic animals (but not from

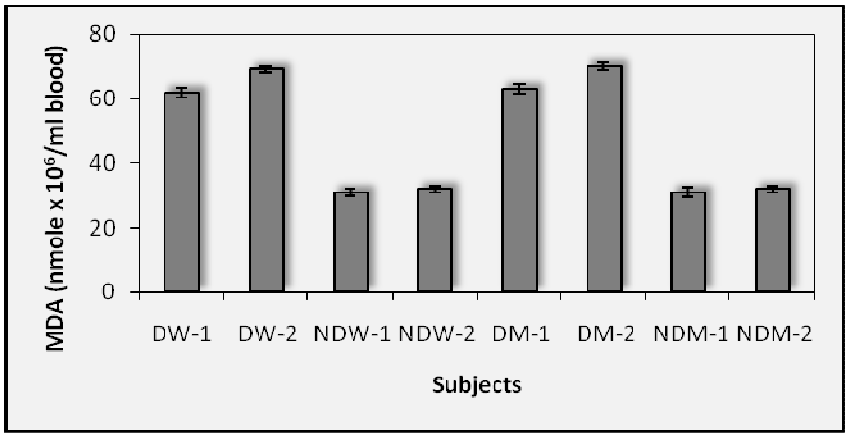
control animals) produce superoxide in the presence of xanthine. This process is inhibited by treatment with heparin which releases xanthine oxidase from the endothelial surface (12).

The activity of XO was also found to be elevated significantly ($p<0.001$) in diabetic subjects above the age 30 when compared to diabetics aging below 30. Age related alteration in XO activity has been reported (11). Aranda et al., (13) have reported an age related positive correlation in ageing and XO activity. Schoutson and de Jong, (14) also have proved an age-dependent increase in XO activity in rat myocytes. In aging, free radicals and other reactive species appear to play an important role in cellular damage and functional degeneration. The accumulation of damaged lipids, proteins, and DNA indicate that the oxidative stress of organism is elevated during aging. Currently, substantial data show that mitochondria are a major source of reactive oxygen species in the aging cells. However, the participation of XOD has received very little attention as a source of free radicals in the aging process (11).

The level of uric acid in diabetics was studied (data not shown). There was no significant elevation in the level of uric acid in both diabetic men and women. Uric acid, the final product of xanthine oxidase has been proposed as a risk factor for coronary heart disease and an independent marker of worse prognosis in patients with moderate-to-severe chronic heart failure.

Free radicals are extremely reactive and consequently short lived, therefore, their activity is usually assessed by indirect methods such as measurement of various end products resulting from interaction of free radicals with cellular components such as lipids, proteins and DNA. Study of interaction of free radicals with lipids can be readily carried out to assess free radical mediated damage. Lipids when react with free radicals, they undergo per-oxidation to form lipid peroxides. Lipid per-oxides decompose to form numerous products including malondialdehyde. Measurement of malondialdehyde levels is the most popular and easiest used indicator of lipid per-oxidation and subsequently free radical reactivity in biological samples. Highly significant ($p<0.005$) levels of MDA was found in the samples of diabetic subjects (Figure 3).

Figure 3. Assay of lipid peroxidation



DW – diabetic women; DM- diabetic men, NDW – non-diabetic women; NDM – non-diabetic men
1 – subjects below 30 years of age; 2 – subjects aged between 30-50 years

Oxidative stress has been considered an important factor in the development of complications in diabetes. xanthine oxidase plays an important role in the generation of free radicals in diabetes. An obvious conclusion is that inhibition of this enzyme should prevent oxidative stress in diabetes. Allopurinol inhibits xanthine oxidase *in vivo*, and it is used in clinical practice. Treatment of patients with allopurinol prevented glutathione oxidation and lipoperoxidation in human type 1 diabetes. Inhibition of xanthine oxidase has proved effective in improving endothelial vasodilator function in hypercholesterolemic, but not hypertensive, patients (12).

The results of the study shows that the activity of XO increases significantly in diabetic subjects of all ages and a marked increase activity of the enzyme was observed with aging. Hence, the study suggests that XO can be a valuable marker of diabetes mellitus.

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