Original Article

Superoxide Radical Concentration and Superoxide Dismutase (SOD) Enzyme Activity in Varicose Veins

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The aim of this study is to measure the level of both the superoxide dismutase (SOD) enzyme and its substrate, superoxide radicals, in the wall of varicose veins. A total of 44 vein specimens were collected from 24 patients who underwent surgery for varicose veins at Asir Central Hospital (ACH), Abha, Saudi Arabia during the period from October 1999 to November 2000. The patients were 4 males and 20 females with a mean age of 35.3±SD 10.4 years (15-62 years). At operation, vein specimens were collected from both the stripped, mid-thigh long saphenous vein (LSV) and the avulsed distal calf varicosities, as appropriate. The samples were processed and both the SOD level and the superoxide radicals concentration were estimated using spectrophotometry. The mean SOD level in the distal calf varicosities (14.7±6.0 units/mg protein) was significantly higher than that in the mid-thigh LSV (8.2±2.9 units/mg protein, P<0.05). The mean superoxide radical concentration in the distal calf varicosities (69.5±11.9 nmol/ml) was also significantly higher than that in the mid-thigh LSV (33.8±10.5 nmol/ml, P<0.05). These results suggest that superoxide radicals play an important role in the pathogenesis of varicose veins. (Ann Thorac Cardiovasc Surg 2002; 8: 286–90)

Key words: varicose veins, superoxide dismutase (SOD) enzyme, superoxide radicals

Introduction

Varicose veins constitute the most common peripheral vascular disorder of the lower limbs. They affect up to 20% of people in the western world and 50% of those over the age of 40 years have some form of varicosity or telangiectasia. Ten percent of affected people develop complications in the form of superficial thrombophlebitis, pigmentation, lipodermatosclerossis, hemorrhage, ulceration and increased risk of deep vein thrombosis (DVT). Because the etiology is unclear, there is no prevention and the problem is increasing.

Several conflicting theories have been proposed to explain the etiology of varicose veins, each attributing their development to a primary factor. Examples of these factors are primary incompetence of the saphenous vein valves,[1,2] inherent weakness of the vein wall,[3] presence of arteriovenous fistulas (AVF),[4] white cell trapping,[5] and so on. The common pathway of these hypotheses is increased pressure in superficial veins with dilatation and secondary incompetence of the valves. The exact cause of the phenomenon remains unclear. On the other hand, epidemiological studies suggest that in addition to genetic factors, several environmental factors such as age, sex, pregnancy and occupation could be associated with the development of varicose veins.[6,7] The impairment in the function of the venous system resulting from these various factors lead to blood stasis and to venous hypertension in leg veins. This is believed to be responsible for the development of varicose veins, since these pathological processes are chronic.[6] The biochemical mechanisms linking blood stasis to the observed alterations in the varicose vein wall is still unclear.

Recently, Michiels et al. studied the effect of hypoxia, usually due to blood stasis, on the interaction between

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polymorphonuclear neutrophils (PMN) and the endothelium, using perfused long saphenous vein (LSV).[8,9] They showed that the adherence of PMNs to the endothelium of hypoxic veins was much stronger than its adherence to that of normoxic veins. The adherence leads to activation of PMNs and possibly increased synthesis of superoxide radicals, which in turn leads to lipid peroxidation and membrane alteration.[10,11] The objective of the present study was to measure the lipid peroxidation as an indication of superoxide radicals concentration in varicose veins, and to correlate this with the activity of the enzyme superoxide dismutase (SOD).

Material and Methods

A total of 44 vein specimens of the LSV and the distal calf varicosities were collected from 24 patients who underwent varicose vein surgery at Asir Central Hospital (ACH), Abha, Saudi Arabia during the period from October 1999 to November 2000. The patients were 4 males and 20 females with a mean age of 35.3±SD 10.4 years (15-62 years). While none of the patients had a history of superficial thrombophlebitis or injection sclerotherapy in the past, 4 patients presented with recurrent varicose veins after previous surgery. Before surgery, all patients underwent both physical and continuous wave Doppler (CWD) examination to assess the extent of their varicosities and the presence of sapheno-popliteal junction (SPJ), saphenofemoral junction (SFJ) and/or LSV incompetence. On Doppler examination, 14 patients had SPJ incompetence, 23 had SFJ incompetence and 21 had LSV incompetence. Based on these findings, patients underwent SPJ and/or SFJ ligation, LSV stripping as appropriate and multiple stab avulsions of the distal calf varicosities. During surgery, vein specimens were separately collected from both the stripped, mid-thigh LSV and the avulsed distal calf varicosities.

The vein samples were washed in normal saline, immediately placed in separate, labeled test tubes and frozen at –20°C, until the time of processing. Samples were later weighed and each 0.5 gm suspended in 4.5 ml of cold phosphate buffer (pH 7.8). Samples were then homogenized for 2 minutes and sonicated at high intensity for 2 more minutes, using a fine probe. The suspension was then centrifuged at 13,000 G for 30 minutes at 4°C. The supernatant was used for both the measurement of lipid peroxidation and the enzyme assay. SOD was assayed at 30°C according to the method described by Misra and Fridovich.[12] One unit is defined as the amount of enzyme, which causes 50% inhibition of epinephrine autooxidation. Protein was determined by the Lowry method.[13]

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production, using thiobarbituric acid (TBA) by the method of Buege and Aust.[14] Lipid peroxides were measured after addition of 2 ml of TBA reagent (15% w/v trichoacetic acid and 0.25 N HCL) to 1 ml of the supernatant. The mixture was then treated in a boiling water bath for 15 minutes. After cooling, the suspension was centrifuged at 1,000 G for 10 minutes. The supernatant was then separated and absorbance was measured at 535 nm. The MDA concentration was determined by the specific absorbance coefficient (1.34×10^5 mol/cm^3).

Mann-Whitney test was used to analyze the test variables, using SPSS®, 7.5 for Windows statistical package.

Results

Out of the 24 patients who were studied in this investigation and underwent varicose veins surgery, only 4 were males with a mean age of 46.0 years. The mean age of the 20 females was 33.1 years (range 15-46 years).

Lipid peroxidation was measured in the wall of 26 vein specimens, 12 from the mid-thigh LSV and 14 from the distal calf varicosities. Comparison of lipid peroxidation in the varicose veins and the mid-thigh LSV is shown in Fig. 1. MDA concentration in the whole group of 26 vein samples was 53±SD 21 nmol/ml. The MDA concentration in the wall of distal calf varicosities was 69.5±SD 11.9 nmol/ml. This was significantly higher than that in the wall of the mid-thigh LSV, which was 33.8±10.5 nmol/ml ($P<0.05$).

In order to correlate lipid peroxidation with the antioxidation status of varicose veins, the level of the enzyme SOD was simultaneously measured. The level of SOD was measured in the wall of 44 vein specimens, 23 from the distal calf varicosities and 21 from the mid-thigh LSV. Concentration of SOD enzyme for the whole group of 44 vein specimens was 11.6±SD 5.8 units/mg protein. Comparison of SOD concentration in the varicose veins and mid-thigh LSV is shown in Fig. 2. The mean concentration of SOD level in the wall of the distal calf varicosities was 14.7±6 units/mg protein. This was significantly higher than the mean level in the mid-thigh LSV, which was 8.2±2.9 units/mg protein ($P<0.05$). Although the lipid peroxidation in the varicose veins was twice that in the mid-thigh LSV, the level of SOD in the varicose veins was only 80% higher than that of the mid-thigh LSV.

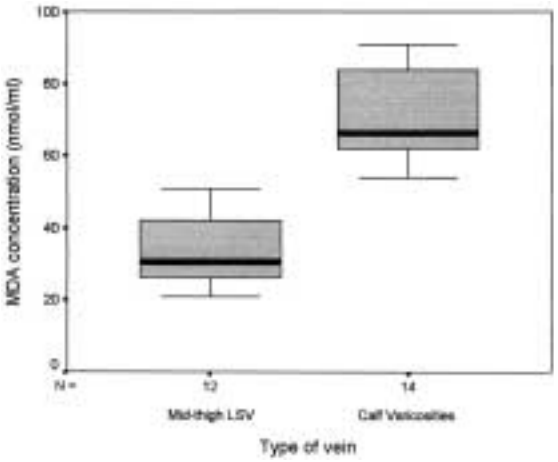

Fig. 1. Box plot showing the difference in the mean values of MDA concentration between the mid-thigh LSV and the distal calf varicosities. (Mann-Whitney test, P<0.05)

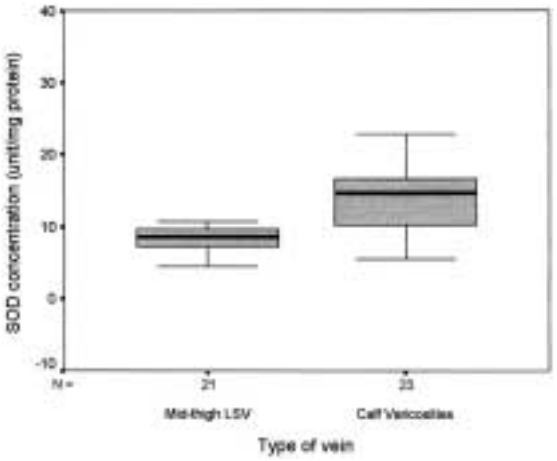

Fig. 2. Box plot depicting the difference in the mean value of SOD enzyme between the mid-thigh LSV and the distal calf varicosities. (Mann-Whitney test, P<0.05).

Discussion

The results presented in this study indicate a few main points. First, most of the patients with varicose veins disease were females. Second, lipid peroxide level, as an indication of the superoxide free radical concentration, in the distal calf varicosities was more than twice that in the mid-thigh LSV. Third, although the level of SOD enzyme in the wall of distal calf varicosities correlated well with the increase in lipid peroxidation, it was only 80% higher in the distal varicosities as compared with the proximal LSV. Lastly, the mean level of SOD for the whole group of veins was almost three times higher than the highest level reported for other tissues in the literature (Table 1).[15]

Varicose veins are said to be one of the most common ailments of people living in Western countries.[16] Although it was thought earlier that varicosis rarely occurs in Asia and Africa,[17] it certainly appears in this part of the world among men and women as shown in this study. It is generally accepted that varicose veins are more common in women,[6,18] which is probably due to the additional factor of pregnancy among them.[19,20] Our study was not intended though to deal with the sexual differences in the occurrence of varicose veins, but it is clear that 83% of the admitted varicose veins patients during the period of this study were females.

There are several conflicting ideas about the etiology of primary varicose veins. Most of these ideas refer to incompetent valves,[21,22] AVF,[4] and defective structure of the vein wall.[23,24] There are also conflicting reports about the content of collagen and elastin in varicose veins. Various authors reported lower content,[25-27] while others reported higher content.[28,29] In an attempt to explain the mechanism of varicosis biochemically, Urbanova and Prerovsky[30] reported significant decrease in the activity of various enzymes including collagenase, elastase, malate dehydrogenase, nonspecific esterases, and 5 nucleotidases, while acid phosphatase and lactic dehydrogenase were significantly increased. Some of these enzymes are involved in the metabolism of collagen and thus could

Table 1. Activity of superoxide dismutase (SOD) in different types of human tissues according to Hartz et al.[15]

Tissue	Unit/mg protein
Liver	4.7
Cerebral cortex	2.9
Testis	2.2
Renal cortex	1.9
Adrenal gland	1.6
Cardiac muscle	1.8
Thyroid gland	1.5
Erythrocytes	2.8
Pancreas	0.5
Spleen	0.5
Lung	0.6
Skeletal muscle	0.5
Prostate	1.1
Bone marrow	Trace

affect its strength. It was also thought that deficiency in prostacyclin (which causes contraction of smooth muscle) might be involved in the development of varicose veins. However, investigators[31] showed higher levels of prostacyclins and thromoboxane A_2 in patients with varicose veins. Recently, Drubaix et a1.[32] advocated the role of glycosaminoglycans, which increase in varicose veins. They also suggested that the decrease in fibrous protein content in varicose veins was probably due to an increased proteolytic or collagenolytic activity or due to the presence of free radicals. Michiels et al.[9] have recently shown that incubation of human saphenous veins in hypoxic conditions lead to the adherence of many neutrophils to the endothelium. These adherent neutrophils were activated and released high amount of superoxide anions and leukotriene B4. These free radicals would be able to degrade the extra cellular matrix and eventually lead to alterations of the vein wall.

Most aerobic cells have an enzyme system to eliminate active oxygen species, because some of these active species are toxic. SOD, catalase and glutathione peroxidase comprise a major defense system against oxygen toxicity. SOD catalyzes the dismutation of superoxide anion (O_2^-) to produce hydrogen peroxide (H_2O_2) and O_2. O_2^- is one of the reduced oxygen species generated in cellular metabolism. Strong evidence exists indicating that many of the cell alterations seen in normal aging and in various diseases, including cancer, are due to oxidative damage from active oxygen species. SOD functions in the cellular defense against the active species, O_2^-. Study of this enzyme is therefore of potential clinical interest. Several laboratories found that in cancer tissues or transformed cells, as well as in aged tissues, the activity of SOD decreased or disappeared as compared to that in uninvolved or younger tissues.[33] SOD has also been related to various disease conditions like diabetes, diabetic retinopathy and cataract, acute myocardial infarction, leukemias, epithelial ovarian cancers and other gynecological malignancies and in primary hepatoma and primary biliary cirrhosis.[33]

In the present study, it is clear that varicose veins have twice the amount of lipid peroxidation compared with the relatively unaffected, proximal thigh LSV. This shows that the level of lipid peroxidation (indicative of superoxide free radicals) correlates very well with the progression of the varicose veins disease, taking into consideration that the distal calf varicosities represent the end-stage of the disease. It is therefore highly possible that the supperoxide radicals are involved in the pathogenesis of varicose veins as suggested earlier by Michiels et al.[9] ln addition, the present study has shown that the level of SOD is likewise significantly increased in the wall of the distal calf varicosities apparently as a protective measure against the increased level of superoxide free radicals. To the best of our knowledge, this is the first study, which clearly correlates the increase of free radicals in the wall of varicose veins with the increase in the activity of SOD, which is a major cellular antioxidant enzyme. Based on these findings, antioxidants could be of help in the treatment of varicose veins to reduce the effect of increasing free radicals. Vitamin E, which is an effective nutritional antioxidant, could therefore be a valuable possibility in the prophylaxis against, or even the treatment of, varicose veins.

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