SEM of Rat Aortae after High Fat Diet and Selenium Supplementation

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**INTRODUCTION**

In the recent experiments in this laboratory, it was found that selenium (Se) supplementation along with high fat diet (HFD) feeding inhibited the induction of hypercholesterolaemia in rabbits and rats [1,2]. Lipid peroxidation and nitric oxide synthase (NOS) activity induced by HFD feeding was also reported to decrease on Se supplementation. On the bases of these studies, the protective effect of Se on HFD induced alterations was concluded to be through its antioxidant action.

Further, there have been a few reports in light microscopic level that Se+HFD group as antioxidant had intensified effect on the reduction of atherosclerotic plaque formation [3]. The present study was undertaken as an attempt to see the various changes occurring in HFD+Se group at the ultrastructural level in aortae using scanning electron microscopy (SEM). Although studies have been published regarding the SEM/TEM appearance of atherosclerotic lesions in aortae from animals [4] fed on HFD as well as man [5], to the best of our knowledge, no study has so far been reported regarding the same in a Se-supplemented group. Our study was aimed at this lacuna in the literature.

**METHODOLOGY**

Treatment protocol: Eighteen Male Sprague Dawley rats (100-125 g bw) were randomly divided into three groups. Group I rats were fed with control basal diet having adequate Se levels (0.2 ppm). Animals in Group II and III were given HFD and HFD+Se (1 ppm as sodium selenite) respectively for 4 months. Diet prepartion is explained in our previous publication [2]. Animals were sacrificed at the end of their diet-feeding schedule by giving them anaesthesia.

Scanning Electron Microscopic Studies: After sacrificing the rat, its aorta was perfusion fixed using 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.2) containing 7.5% sucrose and 0.5% anhydrous CaCl₂. After fixation, the aorta was removed and transferred to fresh fixative, cleared from attached fat. The luminal surface was split open longitudinally, cut into small pieces and again fixed in fixative for overnight at 4°C. After thorough washing in cacodylate, buffer pieces were postfixed in 1% OsO₄ containing 0.05M potassium ferricyanide and 0.5% anhydrous CaCl₂ for 2 hrs at 4°C. Tissue was then washed in triple glass distilled water and stained enbloc in 0.5% aqueous uranyl acetate (pH 3.9) for 20min at room temperature. Dehydration was done in ascending series of acetone (30-95%) at 4°C and finally dehydrated in dry acetone at room temperature. These were then dried in a critical point dryer using CO₂ and acetone a transitional and intermediate fluid respectively. Specimens were mounted on stubs with exposed intimal surface and coated with gold. These specimens were examined immediately under the SEM.

**RESULTS**

SEM of control rats showed normal aortic endothelial surface architecture. All the three layers, i.e. intima, media and adventitia can be well appreciated. Unidirectionally oriented endothelial cells can be seen. Bar =100µm.
face (Fig 2), most of the luminal surface also showed an accumulation of lipid and other cellular debris (Fig 4).

Supplementation of Se along with HFD showed a protective effect of Se on the surface architecture of aortae in comparison to that of the HFD group. The general architecture of the luminal surface was nearly normal showing unidirectional orientation of endothelial cell (Fig 5). Flat endothelial cells with clear margins could be well appreciated (Fig 6). Some adherent cells were present on the aortic surface (not shown), but less than that of aortae from Group II animals.

DISCUSSION
Atherosclerosis is characterised by the focal thickening of the inner portion of the arterial wall in association with fatty deposits. Hypercholesterolaemia has long been accepted as a high risk factor for its development since eating of HFD leads to cholesterol deposition in the arterial wall [6].

Both the etiology as well as the preventive aspects of atherosclerosis are important areas of research. Wojcicki et al [3] has reported the protective action of Se (an antioxidant) against atherosclerosis. Some studies have also indicated the association of Se deficiency with coronary artery disease [7,8]. Our present attempt focused on SEM findings in HFD+Se supplemented rats is presented here. There were marked alterations in SEM findings from aortae of HFD rats versus controls. However Se supplementation led to more or less normalisation of the altered effects of the HFD feeding. The study on the whole depicted the ability of Se, an essential trace element and potent antioxidant, to inhibit the onset of the progression of the disease.

REFERENCES