Effect of vitamin C on endothelial dysfunction during N-α-tosyl L-arginine methyl ester [TAME]-esterase induced contractions in rat aorta in vitro

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Constrictions induced by TAME-esterase on rat aorta strips mounted in vitro were significantly inhibited in presence of Vitamin C. The work lends support to the role of ascorbic acid in preventing endothelial dysfunction through release of nitric oxide. It is suggested that conclusions TAME-esterase could be an important biological marker associated with onset of vascular diseases such as hypertension.

In normal healthy individuals plasma ascorbic acid (Vitamin C) circulates at concentrations of 30-60 μM. However oral supplementation of Vitamin C can almost double the plasma concentrations. Different retention processes that include renal absorption as well as uptake by the intestine, maintain optimal ascorbate concentrations in the plasma. Ascorbate has been reported to facilitate endothelium-dependent vasodilation. Given the prevailing notion that endothelial dysfunction could be an important underlying mechanism for onset of atherosclerosis, a role for oral ascorbate supplementation has been advocated to prevent the disease. Different mechanisms have been proposed by which ascorbate could possibly mediate anti-atherosclerotic effects and the one that retains much attention involves ability of NO to scavenge superoxide radicals within endothelial cells and spare eNOS from oxidative modification. Ascorbate has also been reported to enhance NO-induced vasodilatation of rat coronary and human mesenteric artery segments. It was proposed that effect of ascorbate was due to redox regulation of guanylate cyclase in smooth muscle cells.

N-α-tosyl L-arginine methyl ester [TAME]-esterase has been reported to induce contractile properties in vitro. TAME-esterase has also been described to a possible new cardiovascular risk factor among smokers. Existing data show that calcium channel blockers such as verapamil and nifedipine mimic in reversible fashion effects of Ca²⁺ withdrawal on muscle excitability during TAME-esterase induced contractions on rat aorta in vitro. Available clinical and experimental evidence strongly favours the hypothesis that an impaired pharmacological response of the kinin kallikrein system could increase vasoconstriction and cause a rise in blood pressure (hypertension). The present study has been undertaken to investigate possible effects of Vitamin C on TAME-esterase induced contractile responses in vitro.

Strips of thoracic aorta from Sprague-Dawley rats were mounted in organ bath as described by Gurib and Subratty. Five series of experiments were designed and performed as below.

Group 1: Effects of N-α-tosyl L-arginine methyl ester [TAME]-esterase—For studying the effect of TAME-esterase on aortic strips, a 10^{-1} M stock solution of TAME was prepared by dissolving 0.38g of TAME (Sigma, U.K) in 10 ml distilled water. Aliquots of stock solution were used to make serial dilutions ranging from 10^{-1}-10^{-15} M respectively. Aorta strips (12) were used in this series of experiments. Each strip was challenged with 100 μl of TAME, beginning with the lowest concentration (10^{-15} M). The procedure was repeated in order of increasing concentration to establish a cumulative dose-response curve after stabilization of aorta strip following any contractile responses or after 3 min in case of no observed changes. Final concentration in organ bath was of 4.4×10^{-14} M.

Group 2: Effects of Vitamin C on isolated aorta strips—In this series of experiments, aortic strips were challenged with different dilutions of 100 μl Vitamin C ranging from 10^{-15}-10^{-14} M. Vitamin C was purchased from the Mauritius Pharmaceutical Company.
Group 3: Effects of TAME-esterase on aorta strips pre-incubated with Vitamin C—[TAME]-esterase induced contractions were studied on aorta strips pre-incubated with 500 μl of molar solution of Vitamin C (M). Final buffer concentration in bath was 0.02 M.

Group 4: Effects of Vitamin C on aorta strips pre-incubated with substrate for TAME-esterase—Effects of Vitamin C were studied on aorta strips preincubated with 500 μl of TAME (10^{-1} M), giving a final concentration of 2.3 \times 10^{-3} M.

Group 5: Control experiments—In each series of experiments, a parallel control strip was included and challenged with 100 μl of distilled water added at 3 min interval.

Statistics—All results are expressed as mean ± SE. All data manipulation and statistical analyses were done using Excel software. Statistical differences between means were assessed by one-way analysis of variance (ANOVA). Two way ANOVA was used for analyzing differences between two concentration-response curves. Once a significant difference was detected, Student’s t-test was used to determine enzyme inhibitor concentration at which significant differences were present. P<0.05 was considered to be statistically significant.

Contractile responses induced by TAME-esterase alone or strips pre-incubated with Vitamin C and challenged with the substrate, TAME are expressed as a percentage of maximal response (T_{max}) observed for aorta strips. Curves relating response as a percentage of maximum contractions against logarithm of cumulative concentrations were plotted (Fig. 1). From graphs, EC_{50} values (effective concentration of pharmacological agent producing half the maximal response) were calculated by linear interpolation for each pharmacological agent used on rat aorta strips from respective cumulative concentration curves. [TAME]-esterase induced contractions were significantly inhibited (P<0.05) in rat aortic, pre-incubated with Vitamin C (Table 1). However no significant contractile responses were observed in aorta strips pre-incubated with TAME (10^{-1} M) and challenged with Vitamin C (10^{-12}-10^{-1} M).

This study demonstrated that Vitamin C significantly inhibited TAME-esterase induced contractions in vitro. Intravenous ascorbate infusions or oral ascorbate supplements enhance endothelium-dependent vasodilation through release of eNOS\(^5\). Release of TAME-esterase leads to endothelial dysfunction in rat aorta in vitro\(^8\). Based on present findings it is proposed that in presence of Vitamin C, TAME-esterase induced contractions are significantly subdued due to inability of endothelium to produce adequate amounts of bioactive nitric oxide. The present results are in agreement with the findings that ascorbic acid is one of the most important antioxidants, sparing other oxidants by forming the first line of defense against free radicals and peroxides that are generated during cellular metabolism\(^7\).

Ascorbate may preserve endothelial NO by reducing nitrite to NO could be a plausible mechanism by which Vitamin C could prevent endothelial dysfunction. However, the extent to which this occurs in vivo needs to be established. It is possible that in absence of Vitamin C, release of [TAME]-esterase leads to endothelial dysfunction and causes an increase in concentration of nitrite. In presence of Vitamin C, ascorbate reduces excess nitrite back to NO and hence prevents inhibition of nitric oxide-cGMP mediated pathway\(^7\).

To conclude the present data add on to increasing evidence that TAME-esterase is an important biochemical marker that could be associated with the onset of vascular disease including hypertension and the presence of Vitamin C preserves endothelial NO
generation by diminishing oxidative stress in endothelium.

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References