Effect of genistein, a tyrosine kinase inhibitor, on TAME-esterase induced contractions in rat aorta in vitro

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Effect of genistein, a protein tyrosine kinase inhibitor, on TAME-esterase induced contractions were studied on rat aorta strips in vitro. Data showed that TAME-esterase induced contractions were concentration dependent and these contractions were significantly inhibited when rat aorta strips were pre-incubated with genistein. The present findings provide evidence for the possible contribution of tyrosine kinases during TAME-esterase induced contractions in aorta.

Genistein, a soy derived isoflavone, is a potent prophylactic and therapeutic agent for the treatment of cancer and other chronic diseases\(^1\). Interest that has focused on genistein led to identification of numerous intracellular targets of its action in the living cell\(^2\). At the molecular level, genistein inhibits the activity of ATP utilizing enzymes topoisomerase II as well as enzymes involved in phosphatidylinositol turnover. Moreover, genistein can act via an estrogen receptor-mediated mechanism\(^3\). Genistein, a tyrosine kinase inhibitor, has been widely used to examine potential effects of protein tyrosine kinase (PTK)-mediated regulation of receptor/channel function. Alteration of ion channel function in the presence of genistein has led to the conclusion that PTK regulates the activity of voltage-gated K+ channel under investigation\(^4\). It has been assessed the possibility that genistein directly inhibits glycine receptor, independent of effects on protein tyrosine kinase. Simultaneous application of genistein with glycine reversibly inhibited strychnine-sensitive, glycine-activated current recorded from hypothalamic neurons\(^5\). Time course of genistein action was rapid (within min). Equilibration of genistein in intracellular solution did not affect the ability of extracellularly applied genistein to inhibit glycine response. Glycine concentration-response profiles generated in absence and presence of genistein indicated that the block was due to non-competitive antagonism. Genistein effects also displayed voltage-dependence\(^6\). Daidzein, an analog of genistein that does not block protein kinases, also inhibited glycine activated current. Co-application of lavendustin A, a specific inhibitor of PTK, has no effect on glycine response. It has been demonstrated that tyrosine kinase inhibitor, genistein, has a direct inhibitory effect on glycine receptors that is not mediated via inhibition of PTK\(^7\). Genistein has also been reported to inhibit tyrosine kinase during light adaptation teleost retina\(^8\) can involve activation of tyrosine kinases.

N-\(\alpha\)-tosyl L-arginine methyl ester [TAME]-esterase has been demonstrated to be an enzyme, which is involved in the sequence of events leading to activation of kinin-kallikrein system\(^7\). TAME-esterase has also been described as a possible new cardiovascular risk factor among smokers\(^8\). We have recently reported that calcium antagonists improved N-\(\alpha\)-tosyl L-arginine methyl ester [TAME]-esterase blunted endothelium-dependent relaxation\(^9\). Furthermore, we have also described the role of vitamin C in preserving endothelial NO generation by diminishing oxidative stress in the endothelium\(^10\).

Our study has further evidenced that [TAME]-esterase is an important biochemical marker that can be associated with the onset of vascular disease including hypertension and our work is the first to demonstrate involvement of both kinin kallikrein system and cyclooxygenase pathway in sequence of reactions leading to contractions induced by [TAME]-esterase\(^11\).

The present study was undertaken to investigate the effect of genistein on TAME-esterase induced contractions in vitro.

Ethical clearance for conducting animal experiments and procedure was obtained from the Faculty Research Committee of the University of Mauritius. Male Sprague-Dawley rats were allowed to have
normal diet pellet and tap water ad-libitum. Animals with an initial body weight of 100-220 g were killed by a severe blow to head. Aorta strips were quickly removed as described earlier. The thoracic aorta strip was isolated and mounted in an organ bath containing 25 ml of Krebs-Henseleit buffer of following composition (mM) NaCl, 118; KCl 4.7; CaCl₂, H₂O, 2.5; MgSO₄, 7H₂O, 1.2; KH₂PO₄ 1.2; NaHCO₃ 25; Na₂EDTA 9.7 mg/l and glucose (2g/l). To prevent blood clot formation in the dissected aorta strip, 2 ml of heparin (5,000 IU/L) was added to the buffer in a petri dish. The tissue was maintained at 37°C in a water bath with thermostat and pH of the buffer was adjusted to 7.45. The buffer in the organ bath was oxygenated with a gas mixture of O₂ (95%) and CO₂ (5%). Two stainless steel hooks were inserted into aorta lumen, one was fixed while the other was connected to a force transducer. Isometric contractile responses were recorded with the force transducer (Rikadenki Model R50; Japan) connected to a multichannel recorder (Rikadenki Model R50; Japan). Aorta strips were allowed to equilibrate in the medium for 20 min and maintained under an optimal tension of 2g.

Effects of N-α-tosyl L-arginine methyl ester [TAME]-esterase on rat aorta strips—For studying the effects of TAME-esterase (Sigma, UK) on aortic strips, stock solution of TAME (10⁻¹ M) was prepared by dissolving 0.38g of TAME in 10 ml of distilled water. Aliquots of this stock solution were used to make serial dilutions ranging from 10⁻¹⁵ to 10⁻¹ M respectively. Twelve aorta strips from 12 rats were used in this series of experiments. Each strip was challenged with 100 µl of TAME, beginning with the lowest concentration (10⁻¹⁵ M). A cumulative dose-response curve was established for TAME (10⁻¹⁵ to 10⁻¹ M) after stabilization of the strip. The contractile response was recorded at 3 min intervals when no changes occurred. The final concentration of TAME in the bath was 4.4×10⁻⁴ M.

Effects of genistein on [TAME]-esterase induced contractions on rat aortic strips—In this series of experiments, aortic strips from seven rats were pre-incubated with 500 µl of genistein (1mM) for 20 min before being challenged with 100 µl of TAME (10⁻¹⁵ to 10⁻¹ M), beginning with the lowest concentration.

Control experiments—In each series of experiments, a parallel control strip was included. Aorta strips were challenged with 100 µl of buffer solution was added at 3 min interval for investigating effects of TAME-esterase. However, a parallel control strip was included for the effects of genistein on TAME-esterase induced contraction. Different concentrations of TAME (10⁻¹⁵ to 10⁻¹ M) were added 100µl in cumulative manner and the contractile responses were recorded. Two-way ANOVA was used for analysing the difference between two concentration response curves. Once a significant difference was detected, Student’s t test was used to determine the enzyme inhibitor concentration at which significant differences were present. P values less than 0.05 were considered as statistically significant.

Results of the present study demonstrated that TAME-esterase had a dose-dependent effect causing contractions of aorta strips in vitro. Contractile responses of either TAME alone, or TAME incubated with genistein (1mM) were expressed as a per cent of maximal response (T_max) observed for aorta strips. Curves relating response as a per cent of maximum contraction against logarithm of cumulative concentrations were plotted. From the graphs, EC₅₀ (effective concentration of pharmacological agent producing half the maximal response) was calculated by linear interpolation for each pharmacological agent used on the rat aorta strips from respective cumulative concentration-response curves. Our results (Fig. 1) showed that TAME-esterase induced contractions were significantly inhibited when rat intact aorta strips were pre-incubated with genistein, whereas, in the absence of genistein, a mean (EC₅₀=2.8×10⁻¹⁴ M) was obtained for TAME-esterase induced contraction in rat aorta in vitro.

TAME-esterase induced contractions in vitro have been found to be mediated through a NO-cGMP pathway. Contractile response has also been demonstrated on rat aorta strips, rabbit trachea strips, and frog ileal strips. Our previous work has demonstrated that TAME-esterase induced contraction is blocked by COX-1 inhibitors of cyclooxygenase and ACE inhibitors, vitamin C, and calcium antagonists also block TAME-esterase induced contractions in rat aorta in vitro.

Present work has led us to propose a further possible mechanism whereby second messenger generated during TAME mediated signaling that led to contractions. The possible reason may be — (a) when a receptor is activated by the binding of ligand [TAME] on outer surface of plasma membrane, the receptor-ligand complex associates with the G-protein Gq during activation by phospholipase C (β), causing dis...
Fig. 1—Typical tracings showing [TAME]-esterase induced contraction on rat aorta strips (a) before; (b) after treatment with genistein; and (c) response to 100 µl water.

In conclusion, our results suggest that TAME-induced contractions could involve receptor tyrosine kinases, which in turn can activate phospholipase C gamma which eventually lead to IP3 production followed by release of calcium from intracellular stores causing contraction of the aorta strip.

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References
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