Vascular endothelial growth factor, soluble fms-like tyrosine kinase 1 and genistein-induced changes in the vascular reactivity of rat’s aorta

Anne R. Fernandez and Ruby Husain

Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Abstract

Aim: During preeclampsia (PE), the excessive circulation of soluble fms-like tyrosine kinase 1 (sFLT1) hinders the vasodilatory effect of vascular endothelial growth factor (VEGF). This effect has been proven in vitro in the renal artery of rats. The endothelium of the blood vessels is also said to be dysfunctional in PE. Genistein has shown the ability to antagonize the vascular contractions caused by a wide range of contractile agents. We conducted vascular reactivity studies to demonstrate the effect of: (i) sFLT1 on the vasodilatory effect of VEGF; and (ii) genistein on the vasodilatory effect of VEGF and its effects on denuded blood vessels (dysfunctional endothelium).

Material and Methods: Isolated aortas of male Sprague–Dawley rats were exposed to sFLT1 or genistein and then subjected to increasing doses of VEGF.

Results: The presence of sFLT1 inhibited the vasodilatory effect of VEGF in the rats’ aortas. Genistein significantly potentiated the vasodilatory effect by the VEGF.

Conclusion: The results suggest that genistein may help overcome the vasospasm in PE. It may be a promising therapeutic approach to PE.

Key words: genistein, rat’s aorta, soluble fms-like tyrosine kinase 1, vascular reactivity, vascular endothelial growth factor.

Introduction

Genistein is the most prevalent isoflavone found in soya beans, lentils and legumes. It falls under a larger group of compounds called phytoestrogens. Genistein has been shown to have the ability to antagonize vascular contractions with a wide range of contractile agents, and reduce resistance in a number of arteries in vitro, such as the rat mesenteric artery, aorta and renal artery. Through in vitro experiments, Mishra et al. found that genistein was able to cause relaxation of a rat’s aortic artery irrespective of the presence of endothelium.
of endothelium-dependent vasorelaxation. In vitro experiments have also proven that the serum of women with PE consists of substances that are cytotoxic to the endothelium. VEGF is one of the factors responsible for vasorelaxation during pregnancy. In PE, sFLT1 leaks into the maternal circulation from the ischemic placenta and it binds to the circulating VEGF, preventing it from binding to its actual receptor. The imbalance in the vasoconstrictor and vasodilators leads to a general arteriolar vasoconstriction (vasospasm), which manifests as maternal complications of PE. It has been suggested that overwhelming the action of the vasoconstrictors may be a promising therapeutic approach in PE. In this study, we investigated if the presence of genistein may improve the vasodilatory effect of VEGF.

Methods
The experimental procedures used were approved by the Animal Care and Use Committee, Faculty of Medicine, University of Malaya.

Animals
Male Sprague–Dawley rats weighing 250–320 g were obtained from the Experimental Animal Care Unit, University of Malaya. The rats were provided normal commercial rat chow (Gold Coin Animal Feed, Malaysia) and tap water ad libitum.

Preparation of rat aortic rings
The method for the aortic ring preparation was adapted from Gonzales et al. and Ramachandran. The rats were sacrificed by exsanguination. The aorta was quickly and carefully dissected, removed and immersed into a Petri dish containing oxygenated (ice-cold Krebs-Henseleit [K-H] solution of the following composition [in mM]: NaCl, 120; KCl, 4.7; KH₂PO₄, 1.2; NaHCO₃, 25; CaCl₂, 1.4; MgSO₄, 1.2 and glucose, 10). The adherent fat and excess connective tissues were removed by using a pair of fine scissors, and the aorta was cut transversely into rings of 2–3-mm width while immersed in the K-H solution. The tissue was suspended by two stainless steel hooks in a water-jacketed bath containing 10 mL K-H solution that is maintained at 37°C and bubbled continuously with 95% oxygen and 5% carbon dioxide. Tissues were allowed to equilibrate for 45 min in Kreb’s solution, which was changed every 15 min at a resting tension of 1 g.

Prior to the experiments, all the aortic rings were challenged with KCl solution (60 mM) twice for 5 min. This was done to ensure viability and stabilization of the ring. In some rings, the endothelium was intentionally removed by inserting the tip of fine forceps into the lumen of the aorta and gently rolling it against a wet paper towel. To verify the intactness of the endothelium, the response to the acetylcholine (ACh; 1 × 10⁻⁵ M) was assessed after precontraction with phenylephrine (Ph; 1 × 10⁻⁷ M). The endothelium-intact aortic rings demonstrated more than 70% relaxation while the denuded aortic rings showed minimal (up to 10%) or no relaxation to the ACh that was used in the experiments.

Effects of VEGF, sFLT-1 and genistein in endothelium-intact and -denuded aortic ring
Human recombinant VEGF-165 (VEGF; 0.1–300 ng/mL), mouse sFLT1-Fc (sFLT-1; 1–300 ng/mL) and genistein from glycine max (4′,5,7–trihydroxyisoflavone) (genistein; 1 × 10⁻⁵ to 30 × 10⁻⁵ M) were added cumulatively into the organ bath and dose–response curves were generated for each reagent. The vascular reactivity effect was investigated using the equivalent volume of solvent (dimethyl sulfoxide [DMSO]) used in dissolving the genistein. The endothelium-intact and denuded vascular rings were studied in a paired manner.

Effects of VEGF on aortic ring in the presence of sFLT1 or genistein
The aortic rings were incubated in sFLT1 (30 ng/mL) or genistein (5 × 10⁻⁵ M) for 10 min before being constricted with PE and then subjected to cumulatively added increasing doses of VEGF (0.1–300 ng/mL). The aortic rings were studied in a paired manner.
Chemicals and drugs
Both genistein from Glycine max (4′, 5, 7–trihydroxyisoflavone) and mouse sFLT1-Fc were purchased from Sigma–Aldrich (Malaysia). Recombinant human VEGF-165 was purchased from Shenandoah Biotechnology (USA). All chemicals and solvents were of analytical grade.

Statistical analysis
All results are expressed as mean ± standard deviation with n indicating the ring segments from different rats. Relaxation responses were expressed as percentage of inhibitions of the contraction induced by Ph. Statistical comparisons of percentage of relaxation between groups were performed by two-way ANOVA using SPSS. A P-value of less than 0.05 was accepted as being statistically significant.

Results
Effects of VEGF in endothelium-intact and -denuded aortic ring
Increasing doses of VEGF (0.1–300 ng/mL) induced a statistically significant (P < 0.05) concentration-dependent relaxation in the endothelium-intact aortic ring segments compared to the endothelium-denuded aortic ring segments (Fig. 1).

Effects of genistein sFLT1 VEGF

Effects of genistein in endothelium-intact and -denuded aortic ring
Genistein (1 × 10^{-5}–30 × 10^{-5} M), however, induced concentration-dependent relaxation in both the endothelial intact and the denuded aortic ring (Fig. 2). However, the overall relaxant responses were significantly attenuated in the endothelium-denuded aortic rings (P < 0.05). The DMSO-treated aortic rings showed no significant effects on vascular dilation (data not shown).

Effects of sFLT1 in endothelium-intact and -denuded aortic ring
The sFLT1, on its own, does not cause significant vasoconstriction in endothelium-intact rings. However, it was interesting to note that sFLT1 (30 ng/mL) caused a modest vasodilation of up to 23 ± 3.3% in the endothelium-denuded rings under basal conditions. This effect was not statistically significant.

Effects of VEGF on aortic ring in the presence of sFLT1
In the aortic rings pre-contracted with Ph (10^{-7} M), the VEGF caused relaxation in a concentration-dependent manner with a maximum relaxation of 52.3 ± 17.7% at 300 ng/mL. The presence of sFLT1 at a dose of 30 ng/mL significantly attenuated the vasorelaxatory effect of the VEGF (P < 0.05; Fig. 3).

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Effects of VEGF on aortic ring in the presence of genistein

In these series of experiments, the aortic rings were exposed to genistein before being subjected to increasing doses of VEGF. Genistein at $5 \times 10^{-8}$ M significantly potentiated the overall vasorelaxatory effect by the VEGF ($P < 0.05$; Fig. 4).

Discussion

The present study confirmed that the presence of sFLT1 inhibits the vasodilatory effect caused by the VEGF. This is the first study to report such an effect in rats’ aortic segment. It was also demonstrated for the first time that the presence of genistein enhances the concentration-dependent vasodilatory effect of the VEGF.

The endothelium of the vascular system is a single layer of cells that are essential for the regulation of vascular tone. Endothelium-dependent vasodilation occurs when factors released from the endothelium cause the underlying smooth muscle to relax, thereby dilating the vessel, and is mainly mediated by the release of nitric oxide (NO). Studies have revealed that endothelium-dependent dilator function is compromised in women with PE. In the present study, the VEGF induced a concentration-dependent vasodilation in the endothelium-intact rat’s aorta and this concurs with earlier reports.\(^1\) The vasodilatory effect of the VEGF is endothelium-dependent and a similar effect of VEGF was also found in the uterine and mesenteric artery of rats.\(^1\) Genistein, however, demonstrated a concentration-dependent vasodilation in both the endothelium-intact and the denuded aortic segments. The vasorelaxation effect induced by genistein at a lower concentration was endothelium-dependent whereas the vasorelaxation produced by higher concentrations of genistein were less or not at all endothelium-dependent. This implies that genistein may be able to overcome the effects of the dysfunctional endothelium, which is seen in PE.

In the present study, it was also demonstrated that the presence of genistein enhances the concentration-dependent vasodilatory effect of the VEGF. Contrary to these findings, Szukiewicz et al.\(^1\) proved by using human placental vessels that the effect of VEGF was inhibited by the presence of genistein. In their experiments, the vessels were pretreated with 10-μM genistein and then subjected to VEGF. It is rather difficult to judge the inhibitory effect of genistein on VEGF in these experiments because only a single dose of VEGF ($1 \times 10^{-9}$ M) was used. Perhaps a range of doses would have shown a very different effect. In another series of experiments involving the atrial microvessels, the vasodilatory effects of the VEGF were inhibited by the presence of $10^{-5}$ M genistein. VEGF in the range of $10^{-16}$ M to $10^{-10}$ M, which is rather low, were used in those experiments.\(^1\) In addition, the authors went on to explain that one of the limitations of their study was that the microvessels used were obtained from patients with multiple medical problems in addition to coronary artery disease, and the patients were on various medications for the treatment of other
health ailments. The authors admitted that the blood vessels used should have ideally been from patients with just one disease and they also conceded that the multiple factors contributing to the risk of coronary artery disease may be blamed for the alteration of the vascular reactivity in their study. Therefore, the exact mechanism leading to the altered response of VEGF cannot be determined from their set of experiments.

Traditionally, physiologists have divided circulation into two parts: the large conductance vessels and the resistance vessels. Drance et al.20 stated that the conductance vessels also contribute to the resistance of blood vessels. Through these experiments, it is proven that the rat’s aorta, a large conductance vessel, can behave in a similar manner to the renal vessel, a resistance vessel. The small arteries are known to play an important role in the regulation of peripheral vascular resistance. The response of large arteries can differ considerably as compared to that in small arteries.21 Most of the experimental studies done have proven that sFLT1 inhibits the vasodilatory effect of the VEGF in small arteries but this study has proven the same effect in a large artery.

Rat experiments by Henzel and Alsip22 have demonstrated that the coarctation of aorta in the rat mimics the clinical presentation of mild PE in humans. Simply put, the narrowing of the aorta may also contribute to the pathological changes of PE. This study suggests that the FL1 receptors are present on the endothelium of the aorta and when generalized vasoconstriction takes place in PE, the aorta too may be affected.

It is interesting to note that experiments performed by Parenti et al.23 revealed that experimental conditions mimicking events possibly occurring in vascular pathological conditions, such as a severe hypoxia associated with endothelial damage, caused the VEGF to behave in a paradoxical manner: the relaxant response to the VEGF was reversed to a contractile effect in endothelium-deprived preparations. Another similar effect is seen in ACh, which is known to promote the endothelium to release NO and, therefore, cause vasodilation in the healthy endothelium. However, it was found to cause paradoxical vasoconstriction in the unhealthy coronary arteries by acting directly on the smooth muscle.24 The present experiments confirmed that sFLT1 on its own did not cause vasoconstriction. It behaved like a blocker against the vasodilatory effect of the VEGF in endothelium-intact vessels. Although it is well-known that sFLT1 increases in PE, the exact function of sFLT1 is not yet established. An unexpected finding of vasodilation of up to 23% in the endothelial denuded vessels led to the hypothesis that the weak vasodilatory effect of sFLT1 may be a compensatory mechanism of the body. In the event of an endothelial dysfunction of the blood vessel, the sFLT1 acts directly on the smooth muscle to cause vasodilation. However, this warrants further investigation.

In a review paper by Downing et al.25 discussing the potential druggable targets for the treatment of early onset PE, the authors suggested that NO donors are useful for short-term PE management. In another review paper by Rusin et al.,26 genistein and its derivatives were considered as a NO donor. This property of genisten proves useful as a therapeutic agent in a number of cardiovascular diseases, including hypertension.

Endothelial dysfunction is the hallmark of PE. In a review paper, Vladareanu et al.27 elaborated that the endothelial dysfunction seen in PE is due to: increased circulatory cellular fibronectin, factor VIII and thrombomodulin, alteration of flow-mediated vasodilatation and acetylcholine-mediated vasorelaxation; decreased endothelial vasodilators (such as NO, prostacyclin); increased endothelial vasoconstrictors (endothelin and thromboxane); and increased vascular reactivity to angiotensin II. Extrapolation of the results of the present study to what really happens in the body is seemingly difficult, especially as the current findings were observed in isolated blood vessels that were subjected to angiogenic factors only. Further vascular reactivity studies are needed.

Nevertheless, the vasodilator response brought about by genistein can be considered as a means of rescue from the effects of generalized vasoconstriction seen in PE, as it is able to cause vasodilation in the blood vessel regardless of endothelial integrity. In addition, it enhances the weak vasodilatory effect of VEGF.

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Disclosure

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