Grape seed extract induced nitric oxide mediated endothelial dependent relaxation through AKT/PI3 kinase pathway

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Abstract- A117

Oral administration of grape seed extract (Meganatural®-BP Patent pending) (GSE) lowered the blood pressure in human subjects with the metabolic syndrome. We tested the hypothesis that GSE caused an endothelium dependent relaxation (EDR) in aortic rings from New Zealand White rabbits. The rings were suspended in organ baths containing oxygenated Krebs buffer at 37C. EDR evoked by acetylcholine (Ac) and GSE were measured after pre-contracting the rings with 10-5 M noradrenalin. The tissues were also tested after removing the endothelium and after incubation with L-NAME, Wotmannin (Wot) and LY 294002 (LY) and SU5416 (SU) to examine the mechanism involved in the relaxation. We also investigated the phosphorylation of e-NOS (Ser-1177) by GSE *in- vitro* in HUVECs.

Agonist	Relaxation (%)
Ac (10-5 M)	64 ± 1
GSE (10-3 M)	72 ± 1
Wot (10-7 M) followed by GSE (10-3 M)	7 ± 1
LY (10-4 M) followed by GSE (10-3 M)	6 ± 1
SU followed by GSE (10-3 M)	46 ± 10

Relaxation to GSE was abolished in de-endothelialized and L-NAME treated tissues. The EDR was inhibited by PI3 kinase inhibitors (Wot and Ly) but not by SU (VEGFR2 inhibitor). Involvement of e-NOS was confirmed by phosphorylation of e-NOS (Ser-1177) in HUVECs after exposure to GSE. It is concluded that GSE caused EDR by activation of the AKT/PI3 kinase pathway via a mechanism which does not involve VEGFR2.

Introduction

Several epidemiological studies have indicated that regular intake of vegetables, fruit, and beverages such as red wine and green tea, is associated with a decreased global mortality due to a reduced number of cardiovascular diseases. Grape seed extracts are rich in polyphenolic substances and these compounds are known vasodilators. Our preliminary investigations revealed that administration of GSE significantly reduced the blood pressures in patients with metabolic syndrome and therefore this study was done to investigate the vasodilatory effect of GSE in an *in-vitro* system.

Methods

Grape Seed Extract: The grape seed extract used in these studies is a water extract prepared by Polyphenolic Inc., Madera California (Meganatural B.P.®, Patent pending). The extract is made up of polymers of catechin and has an average degree of polymerization of 2.6. The grape seed extract was dissolved in Krebs Henseleit buffer and the concentrations of the solution were based on a nominal molecular weight of 1000.

Endothelial Dependent Relaxation (EDR): Thoracotomy was performed in white New Zealand rabbits and the descending thoracic aorta excised carefully. The aorta was segmented into rings (5 mm length) and the rings were mounted in standard 15 ml organ baths containing oxygenated (95 % oxygen and 5 % carbon dioxide) Krebs Henseleit buffer (In mmol/l: NaCl 118, KCl 5.4, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 22, NaH_2PO_4 1.2 and glucose 10.1) maintained at 37ºC. A pre-load of 8 g was applied to the rings. The tissues were allowed to equilibrate for 60 min. The transducer was connected to a computerized Gould -2400S recorder system and the changes in tensions were monitored using a Windaq computer program.

After incubating for 1 hr, the aortic rings were pre-contracted with noradrenalin (10^{-5} mol/l). In each animal, all tissues were initially tested with 10^{-5} mol/l acetylcholine. After demonstration of the EDR evoked by acetylcholine, the rings were treated with increasing concentrations of an aqueous extract of acetylcholine or grape seeds (10^{-7}-10^{-5} mol/l) . De-endothelialized tissues were used to demonstrate the endothelial dependent nature of the relaxation. L-NAME, a non competitive eNOS inhibitor was used to demonstrate the involvement of eNOS. We also used the culture human umbilical vein endothelial cells (HUVECs) to demonstrate the GES induced phosphorylation of AKT and eNOS. phosphorylated AKT and eNOS were assessed using immunoblotting techniques in GSE treated HUVEC extracts. VEGFR2 inhibitor (SU5416) was used to see the possible involvement of VEGFR2 receptor on GSE induced EDR mechanism.

Since previous studies have shown that the EDR evoked by certain polyphenolic compounds was abolished by compounds which block the AKT/phosphatidylinositol-3 (PI3) kinase pathway, the responses elicited by grape seed extracts were examined after incubating the tissues with Wortmannin and LY94002 which have been previously shown to block the PI3 kinase. In these experiments rings were first tested with acetylcholine and then re-tested after 30min incubation with the blocker with either acetylcholine or grape seed extract.

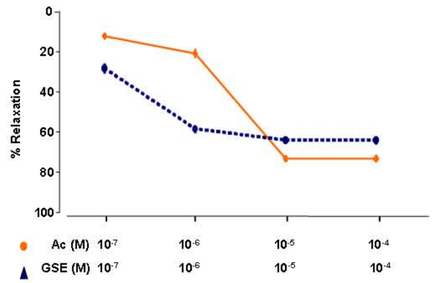
All the experiments shown are the means of at least 5 individual experiments done in different animals.

Hypothesis

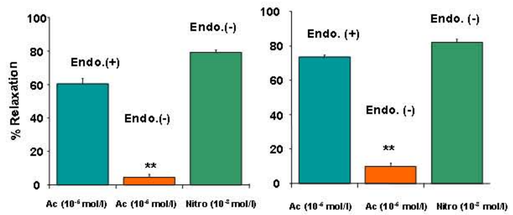
We tested the hypothesis that grape seed extract caused an endothelium dependent relaxation

Results

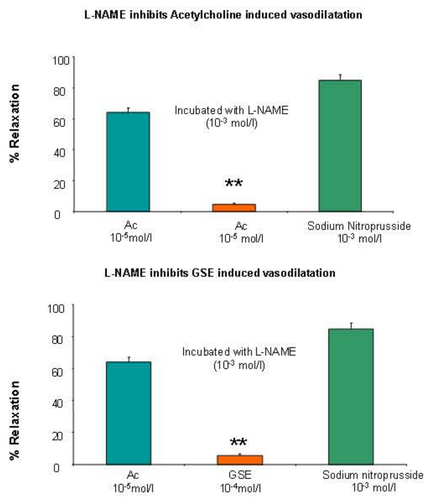
Grape seed extracts caused a dose dependent relaxation in rabbit aortic rings



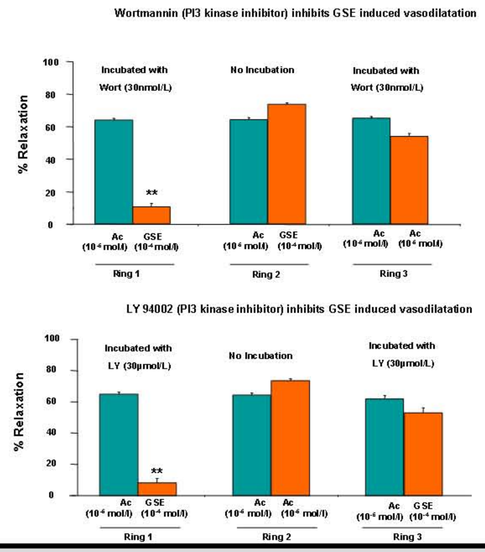
Vasodilatory effect of grape seed extracts in rabbit aortic rings is an endothelium dependent mechanism



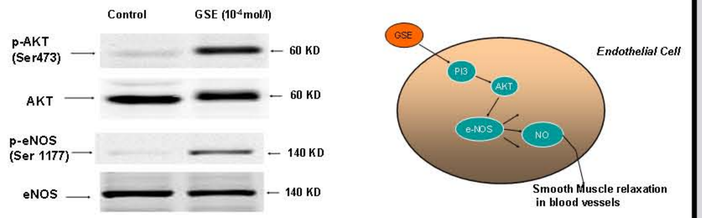
Vasodilatory effect of GSE is dependent on endothelial nitric oxide synthase (eNOS) in rabbit aortic rings



Grape seed extracts induced eNOS through PI3 kinase/ AKT Pathway in rabbit aortic rings



Grape seed extracts caused phosphorylation of AKT and eNOS *in-vitro* in HUVECs



90 % confluent HUVECs were treated with either PBS or GSE (10^{-4} mol/l) for 10 min.

Summary

- Grape seed extract shows dose dependent vasodilatory effect in rabbit aortic rings.
- Relaxation to GSE was abolished in de-endothelialized and L-NAME treated tissues suggesting the involvement of endothelium and eNOS.
- GSE induced EDR was inhibited by PI3 kinase inhibitors (Wortmannin and Ly 29002) but not by VEGFR2 inhibitor (SU5416) suggesting the direct activation of PI3 kinase pathway.
- Involvement of AKT and e-NOS was confirmed by phosphorylation of AKT (Ser 473) and eNOS (Ser-1177) in cultured HUVECs after the cells were exposed to GSE for 10 min.

Conclusions

- **Grape seed extracts elucidated a nitric oxide mediated endothelial dependent relaxation mechanism in rabbit aorta.**
- **The activation of eNOS is a PI3 kinase/AKT dependent mechanism**

References

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