Does ginsenoside treatment in endothelial cells mimic the vasodilatory response induced by G protein-coupled estrogen receptor agonist G-1?

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Background and Rationale

- Atherosclerosis is a progressive disorder characterized by arterial stiffness and endothelial cell (EC) dysfunction.
- Nitric oxide (NO) is a key vasodilator produced by ECs in response to stimulants such as angiotensin II and endothelin-1. NO is produced by endothelial nitric oxide synthase (eNOS), but only after has been phosphorylated at site Ser1177 (p-eNOS).
- G Protein-coupled Estrogen Receptor (GPER) is a seven transmembrane protein expressed ubiquitously by all cells in the body. It has direct vasodilatory effects via NO production.
- The role of GPER in atherogenic EC dysfunction is poorly understood.
- Compounds such G-1 (agonist) and G-36 (antagonist) have been developed to specifically study GPER activation.
- Ginsenoside (Rb1), an active pharmacological ingredient in Ginseng, is known for its vasorelaxant properties.
- The ability of naturally occurring vasorelaxants such as Rb1 to treat endothelial dysfunction and early atherogenesis has yet to be fully explored.

Hypothesis

Signaling through GPER by Rb1 has a vasodilatory response similar to the GPER agonist G1 in endothelial cells.

Objective 1

To conduct a **Literature Review** of the cellular mechanisms controlling GPER-mediated vasodilation in the context of Angiotensin II-dependent endothelial dysfunction.

Objective 2

To perform an **Experiment** to confirm that GPER is expressed by human-derived endothelial cells and to evaluate the response to treatment with G1, Rb1, G36 and their respective vehicles.

Methods

Literature review

- A thorough review of material on the role of GPER in atherogenesis was performed using online databases such as Pubmed® and the journal Atherosclerosis.
- Sources were selected based on their specific focus on GPER's role in regulating processes such as endothelial dysfunction.

Cell culture

- EA.hy926 cells were cultured in 150 mm dishes with 20 mL growth media (DMEM and FBS (9:1)) and incubated at 37°C.
- Medium was changed every 2-3 days.
- Cells were treated when confluent.

Western blot

- **SDS PAGE:** a 6% separating gel and 5% stacking gel were used to separate samples at 20 mA for one hour at room temperature (RT).
- **Gel transfer:** proteins in the gel were transferred to polyvinylidene difluoride membrane at constant voltage (100V) for 1 h.
- **Western blot:** membrane was blocked with 3% BSA in 1× TBST for 2 h at RT. Primary antibody was applied overnight in 3% BSA-TBST at 1:1000 dilution; secondary antibody was applied at 1:10000 dilution for 1 h in 1% BSA-TBST; then washed 4× in 1× TBST after both primary and secondary antibodies were added. After HRP-substrate was added and then imaged using a ChemiDoc™ MP imaging system.

*TBST: tris buffer saline with tween; BSA: Bovine serum albumin; FBS: Fetal bovine serum; DMEM: Dulbecco's Modified Eagle Medium; DMSO: Dimethyl sulfoxide; HRP: horseradish peroxidase; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; DHA: docosahexaenoic acid; AKT: protein kinase B; PI3K: phosphatidylinositol 3-kinase; EGFR: epidermal growth factor receptor; ERK: extracellular signaling-regulated kinase; NADPH oxidase: nicotinamide adenine dinucleotide phosphate oxidase; NFκB: nuclear factor kappa light chain enhancer of activated B cells; ROS: reactive oxygen species; p-eNOS: phosphorylated endothelial nitric oxide synthase: NO: nitric oxide; GPER: G protein-coupled estrogen receptor.

Literature Review Findings

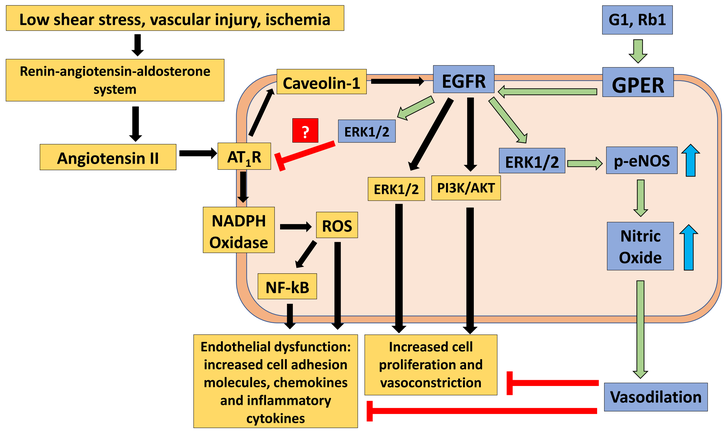

Figure 1. GPER mediated regulation of endothelial dysfunction

- GPER has been identified as a key mediator in promoting and maintaining vasodilation within endothelial cells.
- Vascular injury leads to chronic upregulation of vasoconstrictors such as angiotensin II, which signals through the Type 1 Angiotensin II Receptor (AT_1R).
- AT_1R signaling plays a role in endothelial dysfunction through its activation of NADPH oxidase, forming reactive oxygen species (ROS) which in turn trigger NF-κB activation, as well stimulating MAPK kinases (ERK1/2) through EGFR (Epidermal Growth Factor Receptor).
- ROS and NF-κB increase inflammatory cytokine production and cell adhesion molecule expression, leading to the development of inflammation and stiffening of the arterial walls.
- GPER agonists like G1, and potentially Rb1, have been shown to promote production of the vasodilator NO, and have been suggested to affect AT_1R signaling by desensitizing the receptor.

Experimental Results 1

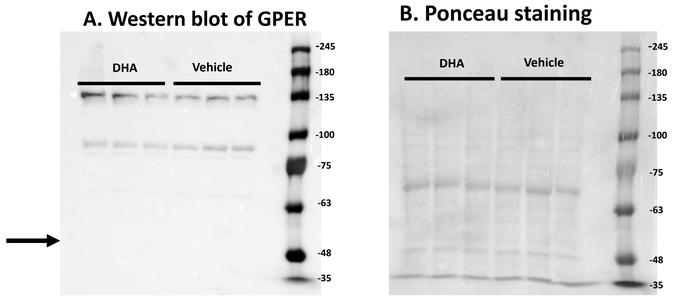

Figure 2. Western blot analysis of GPER expression in EA.hy926 cells with or without DHA (docosahexaenoic acid) for 16 hours.

- Ponceau staining suggests even loading of samples.
- Signaling through GPER by Rb1 has been suggested to exhibit a similar vasodilatory response as the agonist G1. DHA treatment was used to observe if it caused increased expression of GPER.
- As highlighted by the western blot, the expected band at 55 kDa was not observed. Three bands of higher molecular weight were observed instead. GPER was suggested to harbor many glycosylation sites with higher molecular weight in the literature.
- Further work to verify if EA.hy926 expresses GPER is warranted.

Experimental Results 2

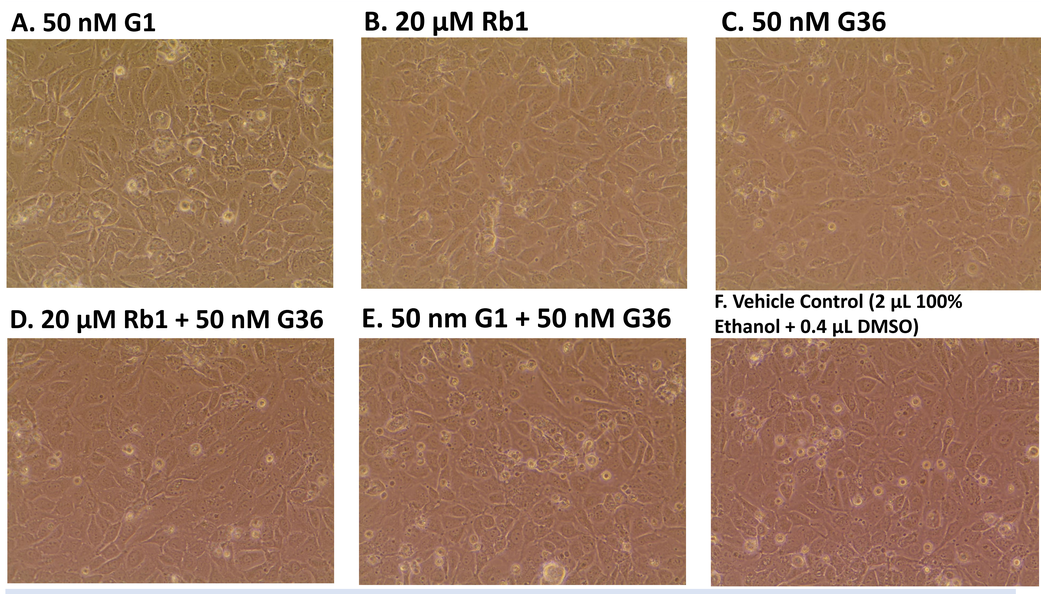

Figure 3. Evaluating the effects of G1, G36, Rb1 and Vehicle on EA.hy926 cell morphology. Samples were treated for 24 hours and then examined using the EVOS XL Core Cell imaging system at 400× magnification.

- Specific concentrations were used based on the literature. All cell treatments displayed no observable signs of toxicity such as abnormal cell morphology or increased numbers of floating cells.

Future Directions

- To perform a time course experiment, using the established concentrations, to evaluate expression of key proteins involved in vasodilation and vasoconstriction, such as eNOS and AT_1R via western blot during 24-hour time period after treatment with vasoconstrictors such as angiotensin II and endothelin-1.
- To determine how long it takes for each treatment to exhibit their maximal effects.

Conclusions

- Based on the literature review, a potential role and signaling mechanism exists for Rb1 to promote vasodilation via GPER mediated signaling.
- G1, G36 and Rb1 exhibited no toxicity; the concentrations are appropriate for future experiments.

Significance

- The significance of these findings include the potential for Rb1 to be further explored as a naturally occurring clinically applicable intervention for early-stage atherosclerosis.
- Further significance includes the potential to better define early atherogenesis in endothelial cells as well as promoting the research of natural relaxants versus synthetically produced compounds.

References

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