Flavan-3-ols, flavonoids, anthocyanidins and triterpenoids induces TIE2 phosphorylation -a candidate target for the vascular protective effects

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Vascular system is essential for the body to maintain health. Dysregulated vascular system leads to cardiovascular diseases and are observed in ischemic stroke, Alzheimer's disease, amyotrophic lateral sclerosis, and diabetes. TIE2 is a tyrosine kinase receptor expressed on vascular endothelial cells and contributes to the maintenance of a vascular system. In this paper, we screened for natural products with an activity to induce phosphorylation of TIE2, which will be beneficial for protection of a vascular system. Employing HeLa cells expressing TIE2, flavan-3-ols, flavonoids, anthocyanidins and triterpenoids were identified as active compounds that induce TIE2 phosphorylation. Several of the identified compounds are previously reported to protect endothelial cells from inflammation. Thus, the result provided TIE2 as the candidate receptor protein of those compounds for the protective effect of endothelial cells and the identified compounds will be a good candidate for maintenance of a vascular system.

Keywords: vascular system; cardiovascular disease; TIE2; catechin; flavonoid; anthocyanidin; triterpenoid

1. Introduction

The vascular system carries blood and lymphatic fluid throughout the body and is essential for the body to maintain health. Dysregulation of the vasculature, including angiogenesis and barrier function, is associated with several diseases. Tumor development is accompanied by progressive angiogenesis (Saharinen et al. 2017). Increased permeability of the vascular system due to dysregulation of the endothelial barrier function leads to leakage of blood components that can cause cardiovascular disease (Saharinen et al. 2017). In ischemic stroke, Alzheimer's disease, and amyotrophic lateral sclerosis, the blood-brain barrier is disrupted (Uemura et al. 2020). The increased leakage is also associated with diabetic complications ; diabetic macular edema is caused by vascular leakage within the eye (Saharinen et al. 2017).

TIE2 is a tyrosine kinase receptor expressed in vascular endothelial cells that regulates vascular inflammation, permeability, and angiogenesis (Saharinen et al. 2017). Binding of the endogenous ligand angiopoietin-1 (ANG-1) to TIE2 induces phosphorylation of TIE2 and activates its downstream signaling factors. It then supports endothelial cell survival, upregulates intracellular adhesion molecules, stabilizes endothelial cell-cell junctions, and protects against inflammation, thereby contributing to the formation of a stable vasculature (Parikh 2017; Saharinen et al. 2017). Therefore, ANG-1 and its receptor TIE2 is a candidate of therapeutic target for preventing vascular problems.

ANG-1 is a potential ligand that activates TIE2, but its application is limited because it is a protein that cannot be applied via oral administration. Furthermore, Ang-1 oligomerizes to obtain its activity, but aggregation also results in poor solubility. The development of cartilage oligomeric matrix protein (COMP)-ANG-1, a soluble and stable ANG-1, solved the latter problem, but oral administration is still limited (Cho et al. 2004). Here, we screened small molecule ligands of TIE2 from nature as alternative candidates for ANG-1 for the prevention and treatment of vascular dysfunction.

2. Results and Discussion

Test compounds were selected from the component of Indian dates (*Tamarindus indica*) (Bhatia et al. 1966; Sudjaroen et al. 2005; Razali et al. 2015; Wandee et al. 2022) and Guava leaf (*Psidium guajava*) (Wang et al. 2010; Chang et al. 2013; Li et al. 2021), which extracts induced phosphorylation of TIE2 (Supplementary Figure S1-S7). The selected compounds were tested against HeLa cells expressing TIE2. Compounds were considered active when Western blotting analysis showed significantly higher p-TIE2/TIE2 value compared to controls. The active compounds found to induce phosphorylation of TIE2, and their minimum effective concentrations are summarized

in Figure S2 (Figure S8,S9 for p-TIE2/TIE2 value and Figure S10-S17 for Western blotting photo).

Of the tested compounds classified as flavon-3-ols, epigallocatechin-3-gallate (EGCG) and gallocatechin-3-gallate (GCG) were active at the lowest concentration (25 μM). Considering the structure of the inactive flavan-3-ols, the 3-*O*-gallate appears to be important for activity. The lower activity of CG and ECG suggests that the phenolic group in the B-ring is important for the activity. In the case of flavan-3-ol dimers, activity was observed for A-type procyanidin and not for B-type procyanidins. However, the B-type procyanidin trimer and tetramer showed activity. This suggests that other factors, such as the spatial arrangement of the flavan-3-ol, rather than the type of bond, may be important.

Among the flavonoids, tricetin and myricetin are active at 50 μM and 200 μM. This indicates that the pyrogallol moiety of B-ring is important. However, the activity of morin at 50 μM indicates that pyrogallol is one of the favourable structures. The lower activity of myricetin compared to tricetin suggest that 3-OH has a negative effect on activity. The negative result of myricitrin indicates that steric hindrance may be a problem.

Anthocyanins had no effect on inducing TIE2 phosphorylation, but anthocyanidins were active. This is a similar trend with flavonoids, where steric hindrance of 3-OH has a negative effect on activity. The number of hydroxyl groups on the B-ring does not appear to strongly affect activity. However, as shown with peonidin, methylation of the hydroxyl group was shown to decrease activity.

Triterpenoids showed activity to induce phosphorylation of TIE2, with the exception of erythrodiol. Maslinic acid (MA) acted at the lowest concentration (12.5 μM). A comparison of the triterpenoid structures shows that the oleanan-type (MA, OA)

is the most suitable, followed by the ursane-type (AA, UA). The lupan-type (BA) showed no activity, indicating that the E-ring is important. The higher activity of MA compared to OA, and AA compared to UA, suggest that the presence of 2-OH has a positive effect on activity. The lack of activity with erythrodiol indicates that a carboxyl group is required for activity.

Among the other tested compounds, casuarinin induced phosphorylation of TIE2 at 5 μM. This was the lowest concentration among the identified compounds. Casuarinin, a type of ellagitannin, have five gallic acids in their structure. Based on the importance of the gallate groups in the activity of GCG and EGCG, it is presumed that the five gallic acids of casuarinin are important for the activity.

Several of the compounds identified as TIE2 activators in this study have been reported to protect vascular endothelial cells. EGCG suppresses inflammation induced by tumor necrosis factor-α in human coronary artery endothelial cells (Reddy et al. 2020), apoptosis induced by high-glucose treatment in human umbilical vein endothelial cells (HUVECs) (Zhang and Zhang 2020), and endothelial barrier dysfunction induced by angiotensin II in glomerular endothelial cells (Bi et al. 2016). The cellular pathways involved in those activities are inhibition of NFκB activation, activation of the PI3K/AKT pathway, and inhibition of p38 MAPK, respectively. Tricetin suppresses oxidized LDL-induced monocyte adhesion to HUVEC through reduced phosphorylation of ERK1/2, increased protein level of EGR-1, and downregulation of intercellular adhesion genes [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] (Cai et al. 2020). Morin inhibits the NFκB signaling pathway in lipopolysaccharide (LPS)-stimulated HUVECs and suppresses inflammatory responses (Meng et al. 2021). MA inhibits TNF-α-induced monocyte adhesion in HUVECs by suppressing NFκB activation and ameliorating increased expression of monocyte

chemotactic protein-1 (MCP-1), VCAM-1 and scavenger receptor type A (SR-A) (Ooi et al. 2021).

ANG-1, a native agonist of TIE2, suppresses LPS-induced inflammatory responses in cultured endothelial cells through suppression of p38 MAPK activation and reduction of NFκB activity (Echavarria et al. 2015). ANG-1 also activates PI3K/AKT pathway to counter VEGF-mediated inflammatory response in endothelial cells (Sako et al. 2009). ANG-1 reduces VEGF-stimulated leukocyte adhesion to HUVECs through reduction of ICAM-1 and VCAM-1 (Kim et al. 2001). A designed ANG-1 variant, COMP-ANG-1 (Cho et al. 2004), induces EGR-1 protein expression in HUVECs (Abdel-Malak et al. 2009).

The action of ANG-1 on endothelial cells and its intracellular signaling pathway is similar to the action and pathway of EGCG, tricetin, morin and MA. These similarities suggest that the protective effects of those compounds on endothelial cells may be induced via TIE2. Thus, the present results provide a candidate receptor for the vasoprotective effects of those compounds. Further studies may reveal the importance of TIE2 in the effects of those compounds.

3. Conclusions

After screening various natural products, several compounds were found to induce TIE2 activation. Since the TIE2 expression system was used to evaluate the activity, its efficacy in vascular endothelial cells needs to be investigated. However, the results do provide a potential receptor for the vasoprotective effects of natural products.

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Declaration of Interest statement

Authors declare no potential conflicts of interests.

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