Do Endothelial cells eat Tryptophan to die?

Charity Duran, PhD and Alejandra San Martín, PhD*
Department of Medicine, Division of Cardiology, Emory University, Atlanta, Georgia 30322

Keywords
NADPH oxidase; endothelial cell; Angiotensin; tryptophan; kynurenine

The endothelium is a vital homeostatic organ fundamental for the regulation of vascular tone and structure. As such, endothelial dysfunction is intimately linked to the development and progression of cardiovascular disease (CVD). The ability of the vasoactive peptide Angiotensin II (Ang II) to induce vascular contractility, endothelial cell (EC) apoptosis and dysfunction via the induction of reactive oxygen species (ROS) is well-appreciated.

Vascular NADPH oxidases (Nox) are predominant contributors to Ang II-induced ROS. In particular, Nox2 and p47phox proteins are recognized to be involved in Ang II-induced hypertension and endothelial dysfunction, which is in agreement with in vitro data showing that the activity of the Nox2-based NADPH oxidase activity from EC, although constitutive, is augmented by Ang II via a mechanism that absolutely requires p47phox phosphorylation and translocaltion to the plasma membrane. Recently, it has also been established that, along with its role in ROS production, Ang II signaling modulates innate and adaptive immunity that critically contributes to the genesis and maintenance of hypertension and vascular dysfunction.

In this issue of Circulation Research, additional pieces of the Ang II signaling pathways are connected as Wang et al. examine a novel mechanism for Ang II-mediated ROS production and vascular dysfunction in vivo involving the crosstalk between interferon gamma (INF-γ) and the kynurenine pathway (Figure 1). The kynurenine (kyn) pathway is a major catabolic route of Tryptophan (Trp), an essential amino acid, infrequently found in proteins. This pathway begins with the oxidation of Trp, which in most cell types is catalyzed primarily by the enzyme indoleamine 2,3-dioxygenase (IDO). IDO, a protein known to be upregulated in response to certain inflammatory cytokines such as INF-γ, produces Kyn, the first stable intermediate in the pathway. Kyn is metabolized in one of two ways: one branch yields kynurenic acid, whereas the other yields 3-hydroxykynurenine (3-OHkyn) and quinolinic acid, precursors of NAD+ (Figure 1). The authors reported that after Ang II treatment, INF-γ induces apoptosis and ROS production in an IDO1-dependent manner. In fact, the deletion of IDO1 alleviates Ang II-induced EC apoptosis and dysfunction by inhibiting superoxide production in vivo (Figure 2).

Interestingly and in agreement with its positive regulation by INF-γ described in the early eighties, experimental evidence has linked the catabolism of Trp to the modulation of immunotolerance. The known fact that INF-γ producing cells are recruited and participate in vascular inflammation raises the interesting possibility that Trp and its metabolites.
participate in the cross talk among different cell types during Ang II-induced hypertension. The mechanism presented could promote a positive feedback loop that maintains and amplifies the effect of Ang II in the EC, or it may serve the opposite purpose, where activation of Trp metabolism within the EC may help to resolve inflammation by reducing Trp in immune cells. This idea is supported by the fact that the cellular stress imposed by local depletion of tryptophan has been shown to induce T cell anergy.

Recent studies demonstrate that the Kyn pathway metabolites are associated with increased oxidative stress, inflammation, and CVD prevalence and atherosclerosis in end stage renal patients. Additionally, Kyn has been identified as an endogenous ligand for the aryl hydrocarbon receptor. Interestingly, a recent report showed that endothelial cell-specific aryl hydrocarbon receptor knockout mice exhibit hypotension and an attenuated response to Ang II.

Downstream Kyn pathway metabolites appear to have differential effects. Ang II induces the production of both Kyn and its metabolite, 3-OHkyn; however, only 3-OHKyn, and not Kyn, induces ROS production and apoptosis in ECs. In other cell types, the expression of quinolinic acid phosphoribosyltransferase (QPRT), which metabolizes quinolinic acid to nicotinamide adenine dinucleotide, suppresses caspase-3, inhibiting apoptosis and in gliomas the induction in QPRT expression positively correlates with tumor malignancy. It could be that the effect of this pathway in the cell cycle is cell type specific, or that in other cell types 3-OHKyn has similar effects as in EC and the increased activity of downstream enzymes actually reduces the intracellular concentration of 3-OHKyn.

In addition, Wang and collaborators reported that the inhibition of kynurenine monooxygenase (KMO) prevents NADPH oxidase-induced ROS generation and INF-γ-induced apoptosis. In particular, the role of (KMO) warrants further study. Because it is the enzyme responsible for the metabolism of Kyn to 3-OHKyn, KMO is a potential target for alleviating Ang II-induced oxidative stress. Interestingly, non-stimulated EC have no KMO basal activity since Kyn is unable to induce apoptosis and thus, most likely is not metabolized to 3-OHKyn in cells that have not been exposed to INF-γ. Therefore, KMO activity does not seem to be required for EC normal function, making it an attractive pharmacological target. Indeed, KMO is already being investigated as a potential neuroprotective target due to its involvement in pathologies of the central nervous system such as neurodegenerative disorders, pain syndromes and autoimmune diseases. While the role of kyneurinine metabolites in oxidative stress and CVD has not been closely examined, Kyn induction of oxidative stress has been more extensively studied within the context of neurodegenerative disorders such as Huntington’s Disease. Both 3-HK and quinolinic acid exhibit neurotoxicity, and can induce oxidative stress and apoptosis in neurons. Another kynurenine pathway metabolite, kynurenic acid, exhibits neuroprotective effects in part due to its ability to scavenge free radicals. It has yet to be determined if kinurenic acid can exert similar protective effects in the context of Ang II-induced inflammation.

Because 3-OHKyn mediated ROS production is attenuated by treatment with either an SOD mimic or apocynin, the authors conclude that the major source of Ang II-induced ROS is NADPH oxidase. Furthermore, 3-OHKyn treatment increases translocation of both p47phox and p67phox to the membrane fraction and increases the fraction of these proteins that are modified by 3-OHKyn-KLH adducts, suggesting that 3-OHKyn regulates Nox activity by promoting modification and the subsequent translocation of these subunits to the complex. As has been discussed extensively in the field, the interpretation of data obtained using apocynin in vascular cells has to be done cautiously and more work is necessary to
determine if the translocation of the subunits to the plasma membrane is in fact a consequence of the direct modification of the phox proteins by 3-OHKyn.

Taken together, the study presented by Wang et al. reveals a novel role for the kyn pathway in mediating Ang II-induced ROS production and EC dysfunction. Even if the contribution of Ang II-induced EC apoptosis to vascular pathology is relatively minor, the involvement of the kyn pathway in Ang II-induced ROS production could modulate a variety of Ang II effects in the vasculature. This, alongside the recent discovery that increased circulating 3-OHK is associated with CVD, opens intriguing new avenues of research.

Acknowledgments

Sources of Funding

The National Heart, Lung and Blood Institute of the National Institutes of Health under awards R01HL113167, R01HL58863 and T32HL007745 support our studies.

References


Figure 1.
Principal metabolic outcomes of the kynurenine pathway.
Figure 2. Proposed model
Circulating AngII stimulates immune cells to secrete INF-γ, which induces catabolism key enzymes of Trp catabolism. The formation of 3-OH Kyn, product of the Trp degradation pathway, induces the modification and subsequent translocaltion of phox cytosolic subunits to the membrane activating NADP oxidase activity. NADPH oxidase-derived ROS reach the mitochondrial where are able to induce release of Cytochrome C and apoptosis.