Biased Agonism of the Angiotensin II Type I Receptor
A Potential Strategy for the Treatment of Acute Heart Failure

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SUMMARY

Angiotensin II (AngII) type I receptor (AT1R) recognizes AngII, a cardiovascular peptide hormone that acts as a terminal effector of the renin-angiotensin system (RAS). AT1R belongs to the rhodopsin-like peptidergic family of G protein-coupled receptors (GPCRs) and serves as a therapeutic target for the treatment of cardiovascular diseases, such as hypertension, cardiac hypertrophy and heart failure. Classically, AT1R was considered to signal only through G proteins. However, recent studies have revealed that AT1R is capable of activating G protein-independent signaling that is mediated by β-arrestins. β-arrestin is a cytosolic scaffold that is recruited to the activated GPCRs. In vitro and ex vivo studies have demonstrated that the activation of the AT1R-β-arrestin pathway stimulates contractility and exerts prosurvival effects in cardiomyocytes. TRV027, a potent synthetic β-arrestin-biased ligand for AT1R, specifically activates AT1R-β-arrestin signaling without stimulating G proteins. In preclinical studies, TRV027 not only produced vasodilation by antagonizing the AT1R-Gq pathway but also enhanced cardiac performance by activating AT1R-β-arrestin signaling. Because of this unique pharmacological profile, TRV027 is now being evaluated in a phase II clinical trial as a novel therapeutic for acute heart failure (AHF). (Int Heart J 2015; 56: 485-488)

Key words: GPCR, β-arrestin, β-arrestin-biased ligand, Novel cardiovascular therapeutic, TRV027

Angiotensin II (AngII) type I receptor (AT1R) belongs to the rhodopsin-like peptidergic family of G protein-coupled receptors (GPCRs) and recognizes AngII (Asp1-Arg2-Val3-Tyr4-Ile5/Val3-His5-Pro6-Phe7), a critical cardiovascular hormone that functions as a terminal effector of the renin-angiotensin system (RAS). RAS plays important roles in the regulation of the cardiovascular system: thus, AT1R is a therapeutic target for the treatment of cardiovascular diseases, such as hypertension, cardiac hypertrophy and heart failure. AT1R antagonists (ARBs) are widely used for the treatment of hypertension. Notably, ARBs have also been demonstrated to exert beneficial effects on cardiovascular organ damage beyond their blood pressure-lowering activity. The high-affinity binding of AngII to the AT1R (Kd: ~1 nM) activates Gq/11 proteins, resulting in intracellular inositol triphosphate (IP3) production, calcium mobilization and protein kinase C activation. These G protein activation-dependent intracellular events have been thought to mediate the physiological effects of AT1R signaling.

Biased Agonism of GPCRs

GPCRs contain a characteristic seven-transmembrane domain. The conformational rearrangement of the 7 transmembrane alpha helices induced by the ligand binding leads to the activation of heterotrimeric G proteins followed by the recruitment of β-arrestins (Figure 1). β-arrestins are cytosolic adaptor proteins originally identified as a desensitizer of G protein-mediated signaling that is recruited from the cytosol to the ligand-bound GPCRs. Interestingly, however, recent studies have found that β-arrestins also act as a scaffolding molecule that mediates G protein-independent signaling (Figure 1).

The G protein signaling pathway and β-arrestin signaling pathway are currently considered to be independent of each other. Consistent with this hypothesis, some GPCR ligands selectively activate either a G protein- or β-arrestin-mediated signaling, which suggests that GPCRs can exist in distinct active conformations depending on the structures of the ligands binding to the receptors. β-arrestin signaling has been shown to have differing physiological effects from those of the classical G protein-mediated signaling, thus indicating that chemical compounds acting on the same receptor may have distinct in vivo pharmacological actions based on varying degrees of bias towards G protein and β-arrestin signaling. This can be applied to AT1R. Rather than inhibiting both the G protein- and β-arrestin-mediated pathways as ARBs (competitive antagonists for AT1R) do, biased ligands for AT1R that differentially activate or inhibit one pathway over the other would be beneficial in particular pathophysiological conditions (Figure 2).
The highly conserved Glu/Asp-Arg-Tyr sequence (DRY motif) in the second intracellular loop of GPCRs has been demonstrated to be important for many GPCRs to interact and activate G proteins. Based on this finding, G protein-independent signaling downstream of AT1R was examined by generating a mutant AT1R receptor (DRY/AAY), which was expected to have impaired Gaq/11-coupling. No increase in IP₃ production was observed when the AT1R mutant (DRY/AAY) was stimulated with AngII. Interestingly, however, this mutant retains the ability to recruit β-arrestins and trigger robust mitogen-activated protein kinase (MAPK) activation upon ligand stimulation. MAPK activation was revealed to be dependent on β-arrestins via an RNA interference technique to knockdown β-arrestins. These results indicate that the AT1R mutant (DRY/AAY) is a β-arrestin-biased receptor that does not activate G proteins but transmits intracellular signals through the β-arrestin-MAPK pathway.

Similar results were obtained when a wild-type AT1R was stimulated with a synthetic AngII analog (SII: [sarcosine¹, Ile⁴, Ile⁸] AngII). The SII ligand was unable to increase IP₃ production but still induced receptor internalization, β-arrestin recruitment and robust MAPK activation. These results suggest that SII acts as a β-arrestin-biased ligand for AT1R.

Thus, although AT1R activates both the G protein- and β-arrestin-mediated pathways following AngII stimulation, AT1R is also capable of selectively activating β-arrestin signaling depending on the ligands involved (Figure 2).

**Figure 1.** G protein and β-arrestin-mediated signaling. β-arrestins were originally identified as a desensitizer of G protein-mediated signaling that is recruited from the cytosol to the activated receptor. However, recent studies have revealed that β-arrestins also act as a scaffolding molecule mediating G protein-independent signaling. These two signaling pathways are considered to be independent of each other.

**Figure 2.** Balanced and biased ligands for AT1R. A: AngII: a balanced endogenous agonist for AT1R. AngII activates both G protein- and β-arrestin-mediated signaling. B: ARBs: balanced antagonists for AT1R. ARBs inhibit both G protein and β-arrestin-mediated signaling. C: SII and TRV027: β-arrestin-biased agonists for AT1R. SII and TRV027 inhibit G protein signaling but activate β-arrestin-mediated signaling.
Physiological Consequences of AT1R-β-arrestin Signaling in the Cardiovascular System

Utilizing SII (a β-arrestin-biased ligand for AT1R) as a pharmacological probe, the physiological consequences of AT1R-β-arrestin signaling were studied. SII was found to induce chemokinesis in HEK293 cells overexpressing AT1R. SII was also reported to exert anti-apoptotic cytoprotective effects and promote protein synthesis in rat vascular smooth muscle cells by stimulating endogenous AT1R in vitro and in vivo. Ex vivo studies further revealed that SII treatment had positive inotropic and lusitropic effects in cardiomyocytes isolated from wild-type mice but not in cardiomyocytes from β-arrestin2 deficient mice. Consistent with the previous in vitro studies, SII was able to activate MAPK in perfused hearts. These results indicate that AT1R-β-arrestin signaling may significantly impact cardiovascular physiology.

Mechanical stretch has been suggested to activate endogenous AT1R in cardiomyocytes without the involvement of AngII release. The mechanical stretch-induced activation of cardiac AT1R resulted in MAPK activation in vitro and cardiac hypertrophy in vivo. Recently, Rakesh, et al. showed that mechanical stretch triggers AT1R trafficking and β-arrestin recruitment in the absence of G protein activation. Mechanical stretch also elicited conformational changes in β-arrestins that are similar to those induced in SII treatment. These results indicate that mechanical stretch leads to β-arrestin recruitment to AT1R without activating G proteins and triggers a conformational change in β-arrestins that augments receptor internalization and intracellular signaling.

Mechanical stress promotes apoptosis in cardiomyocytes, whereas AT1R-β-arrestin signaling has been shown to exert anti-apoptotic effects in vitro. To examine whether AT1R-β-arrestin signaling triggered by mechanical stretch acts as a pro-survival signal, hearts isolated from wild-type mice, AT1aR-deficient mice and β-arrestin2-deficient mice were subjected to balloon stretch ex vivo. Mechanical stretch resulted in Akt phosphorylation in wild-type hearts but not in AT1aR-deficient hearts or in β-arrestin2-deficient hearts. In agreement with this, the rate of apoptotic cell death was significantly enhanced in the AT1aR-deficient hearts and in the β-arrestin2-deficient hearts compared with that in the wild-type hearts, suggesting that stretch-induced AT1R-β-arrestin2 signaling promotes cell survival pathways in cardiomyocytes. Losartan, which is one of the ARBs, can stabilize the AT1R in an inactive state conformation. Surprisingly, losartan treatment resulted in increased apoptosis in hearts subjected to mechanical stretch. These results provide significant clinical implications. Because ARBs are balanced competitive antagonists for AT1R, they inhibit not only the G protein-mediated pathway but also stretch-induced β-arrestin signaling, which is beneficial for promoting cell survival in hearts exposed to mechanical stress. This raises the possibility that β-arrestin-biased agonists for AT1R that inhibit the G protein-mediated pathway but activate β-arrestin signaling could be advantageous in limiting cardiomyocyte injury in cardiovascular disorders (Figure 2).

A knockin mouse model was created with a gain-of-function AT1αR mutant that harbors a constitutively active mutation (N111S) with a C-terminal deletion, resulting in impaired receptor internalization and desensitization due to an inability to recruit β-arrestins. This mouse model exhibited cardiac and renal fibrosis despite a moderate increase in blood pressure (~20 mmHg). A transgenic mice line was also created by overexpressing AT1aR mutant lacking G protein coupling in cardiomyocytes. In contrast, these mice exhibited less cardiac fibrosis and apoptosis compared with cardiomyocyte-specific wild-type AT1aR transgenic mice. Although the direct involvement of β-arrestin signaling was not demonstrated, these in vivo experiments suggest that AT1R-β-arrestin signaling may play a protective role against cardiac fibrosis.

TRV027: Novel Heart Failure Therapeutics Based on Biased Agonism of AT1R

SII, a synthetic β-arrestin-biased ligand for AT1R, induces β-arrestin recruitment, AT1R internalization and MAPK activation without activating G proteins. The unique in vitro and ex vivo consequences of AT1R-β-arrestin signaling activation were evaluated using this ligand. However, because SII is a low-affinity ligand for AT1R, it has been difficult to study whether the in vivo administration of SII elicits distinct pharmacological effects compared with those induced by the administration of balanced ligands, such as AngII and ARBs. To overcome this limitation, potent β-arrestin-biased ligands were identified through an iterative evaluation of peptides that were custom-synthesized based on the SII sequence. Among the identified ligands, TRV027 (Sal-D-Ala2-Val5-Tyr3-Ile6-His9-Pro14-Val20) exhibited improved potency (EC50: 17 nM) comparable to that of AngII (EC50: 9.7 nM) in a β-arrestin assay but no detectable G protein activation. Consistent with the previous findings obtained via SII treatment, TRV027 increased cardiomyocyte contractility in vitro. When administered in rats, TRV027 decreased the mean arterial pressure by inhibiting AT1R-Gqα-mediated vasoconstriction in a manner similar to ARBs. Furthermore, TRV027 increased cardiac performance and preserved stroke volume by activating β-arrestin signaling. Because ARBs are known to decrease cardiac performance in the acute phase, the in vivo actions of TRV027 were strikingly different from those of ARBs. This unique and advantageous pharmacological profile of TRV027 has also been demonstrated in translational preclinical studies using normal dogs and dogs with tachypacing-induced acute heart failure (AHF). In these studies, TRV027 not only reduced pulmonary capillary wedge pressure (PCWP) and renal vascular resistance but also increased cardiac output, indicating that TRV027 is inherently beneficial in the treatment of AHF (Figure 2).

A first-time-in-human trial for TRV027 was performed to test its safety, tolerability, pharmacokinetics and pharmacodynamics in normal volunteers. TRV027 was shown to be safe and well tolerated with a predictable pharmacokinetic profile (a short half-life: 2.4–13.2 minutes). In agreement with the preclinical studies, TRV027 infusion exhibited a reversible dose-dependent decrease in the mean and diastolic arterial pressures. Notably, these blood pressure-lowering effects of TRV027 were observed only in subjects with activated RAS following short-term dietary sodium restriction. This finding provides an intrinsic safety feature: TRV027 would be effective only in patients with the target pathophysiology (ie, activated RAS).

Due to the promising results of the phase I trial, a phase IIa study was conducted in patients with stable systolic heart failure (NYHA class 3 or 4, ejection fraction < 35%, baseline average PCWP ≥ 20 mmHg on 3 consecutive measurements).
Intravenous infusions of TRV027 were found to be safe and well tolerated in this study as well. As expected, the pharmacological effects of TRV027 on the mean arterial pressure and PCWP were apparent in subjects with activated RAS, and the pharmacokinetics of TRV027 were consistent with those observed in preclinical and phase I trials. To determine the dose and study design for further development of TRV027 as an AHF therapeutic, TRV027 is now being evaluated in BLAST-AHF (Biased Ligand of the Angiotensin Receptor Study in Acute Heart Failure; NCT01966601), an international prospective, randomized, dose-ranging phase IIb clinical trial that will enroll up to 500 patients with AHF. 24

Conclusions: Classically, GPCRs were considered to function as a binary switch (ie, on and off) and transmit signals only through G proteins. However, recent studies have revealed that GPCRs are capable of activating two independent pathways (ie, G protein- and β-arrestin-mediated pathways) and adopting more than one active conformation depending on the ligands involved. The concept, referred to as “ligand bias”, states that GPCR ligands exert distinct pharmacological effects based on varying degrees of bias towards the two independent pathways even though the ligands act on the same receptor. Ligand bias has been heavily studied utilizing AT1R as a model GPCR. In vitro and ex vivo studies revealed that AT1R can exist in a conformation that selectively engages with the β-arrestin pathway and that the activation of the AT1R-β-arrestin pathway enhances contractility and prosurvival signals in cardiomyocytes. In preclinical studies, TRV027, a potent β-arrestin-biased ligand for AT1R, has been shown to produce vasodilatation by antagonizing the AT1R-Gαq pathway and enhance cardiac performance by activating AT1R-β-arrestin signaling. Because of this unique pharmacological profile, TRV027 is considered beneficial in AHF treatment. TRV027 is currently being tested in a phase IIb clinical trial (BLAST-AHF) that will randomize up to 500 patients with AHF. Thus, TRV027 provides a promising example for the successful translation of evolving concepts in GPCR research (ie, ligand bias) into innovative medicines.

References

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