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EDITORIAL

Relaxin-2 for heart failure with preserved ejection fraction: a comment on the termination of a phase-II trial investigating the relaxin-2 analogue, LY3540378

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Abstract

On November 25, 2024, Eli Lilly and Company informed the public about the decision to terminate their phase-2 study, J3E-MC-EZDB with the relaxin-2 analogue, LY3540378, after data analysis of 40% of participants had indicated futility without immediate signs of patient risk. The study enrolled patients with heart failure with preserved ejection fraction within 2 weeks of an event of worsening heart failure with volume overload to receive 3 different doses of LY3540378 or placebo as weekly subcutaneous injection. In this Editorial, we compare LY3540378 and related long-acting relaxin-2 analogues with native relaxin-2. It is demonstrated that the modifications to native relaxin-2 to increase its half-life in circulation have been achieved at the cost of i) safety and ii) signalling pathways pivotal to the treatment of HFpEF. In terms of safety concerns, elevated immunogenicity attributed to the fusion to Fc or antibody fragments is forwarded, as well as impairment of therapy control in blood pressure-labile HFpEF patients due to overly prolonged administration intervals. With respect to signalling, we elaborate on the glucocorticoid-receptor and Wnt1 pathways that control anti-inflammatory and anti-arrhythmic therapy effects. It is highly unlikely that those pathways are activated by the long-acting relaxin-2 analogues. Using the Wn1 pathway, native relaxin-2 increases markedly the expression of the fast sodium channel, Nav1.5 in cardiomyocytes from aged rats, to ~200 % after 48 hours. In contrast, increasing doses of a single-chain analogue of relaxin-2, B(7-33) have no effect on Nav1.5. In summary, we make a case for the therapeutic use of full-length, native-structure human relaxin-2 in HF, especially in HFpEF. We need the full pleiotropy of the native peptide for a most complex clinical syndrome.

Key words: heart failure with preserved ejection fraction; relaxin-2; biased agonism; glucocorticoid receptor; Wnt1.

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Has the time come to abandon hope of relaxin-2-derived therapies for heart failure (HF)?

The relaxin-2 analogues under development by Eli Lilly (LY3540378),[2] Astra Zeneca (AZD3427),[3] as well as Tectonic Therapeutic (Tx-45)[4] share a common concept: structural modification of native human relaxin-2 to increase its plasma half-life from below 1 hour[5] to many days. This is achieved by i) sequence modification, ii) change of the native insulin-like, two-chain structure into a single-chain peptide with an artificial short linker connecting A and B chains, and iii) fusion to a single-domain antibody against albumin and an IgG-Fc fragment for the Eli Lilly and the Astra Zeneca / Tectonic analogues, respectively. All these fusion peptides undergo recycling *via* the neonatal F_c fragment receptor-γ (FcRn-γ), which serves to prolong half-life.[6].

For LY3540378, high-affinity binding of the antibody moiety to human albumin (dissociation constant, K_D ~8) additionally contributes to this end.[2] These alterations have two major consequences: higher risk of adverse effects and loss of important signalling pathways that have been attributed to the native peptide.

With respect to adverse effects, one has to consider heightened immunogenicity, which is related both to the alteration of an endogenous peptide and its fusion to antibodies or fragments thereof. First, the joining region of fusions is known to potentially elicit immune responses due to neoantigen formation.[6] For LY3540378, in addition, it is the variable part of a heavy-chain antibody against human albumin that may bear immunogenic potential. Eventually, F_c fragments as incorporated in

AZD3427 and Tx-45 are generally considered to cause canonical and non-canonical F_c fragment receptor-γ- (FcR-γ-) mediated effects, such as antibody-derived cell cytotoxicity, complement activation, phagocytosis, or activation of antigen-presenting cells.[6]

Another concern about safety arises from the pronounced susceptibility of HFpEF patients towards preload and afterload changes causing great blood pressure lability, which may translate into worsening of renal function or hypertensive crises.[7] The half-lives of the relaxin-2 analogues have been increased manifold over that of the native peptide. Administered as sc. injection, LY3540378 is given once weekly, AZD3427 every 2 weeks, and Tx-45 even once monthly. While this may be convenient, it elevates the risk of hypotension as relaxin-2 is a potent vasodilator.[8] Shorter dosing intervals would offer better therapy control. Even more importantly, it remains obscure if the considerably larger relaxin-2 analogues still have access to the central nervous circumventricular organs. Those are the brain regions where circulating native relaxin-2 elicits central angiotensin-II and vasopressin increases. This pressor effect serves to compensate for vasodilation and spans what we may call a blood pressure safety net.[9,10]

In regard to signalling, the cognate receptor for relaxin-2, relaxin family peptide receptor-1 (RXFP1) is a highly complex type-C leucin-rich repeat-containing G protein-coupled receptor.[8,11] Despite ongoing progress, full structural details of how relaxin-2 binds RXFP1 and elicits, in a multi-step process of successive conformation changes, signalling activation are still lacking.[11] In addition, pathways activated by relaxin-2 vary extremely between different types of native receptor-expressing cells,[8] as well as between native-expressing and RXFP1-overexpressing cells: B(7-33) (B7), a research-only single-chain derivative of human relaxin-2, was shown to be a low-affinity RXFP1 binder (B7, pK_i [negative decadic logarithm of inhibition constant] 5.5; relaxin-2, pK_i 9.0) as well as a weak cAMP agonist in RXFP1-overexpressing HEK-RXFP1 cells, but was fully active in human cardiac fibroblasts as compared to the native peptide.[12]

What does this have to do with the termination of LY3540378 development? The meticulous up-engineering of half-life may have been achieved at the cost of essential signalling pathways – current relaxin-2 mimetics may not really mimic the native peptide's complex and highly beneficial spectrum of effects. Second, we need testing in natural cells and, preferably, in the respective disease models as there is no such thing as canonical signalling for relaxin-2. Two examples shall be given:

Human relaxin-2 is a very potent agonist (K_D ~9) at glucocorticoid receptor (GR),[13] and a relevant part of its anti-inflammatory, anti-oxidative and vasoprotecting effects is attributable to GR.[13,14] This spectrum is highly desirable for HFpEF, which is a syndrome where the heart and vessels fall victim to ongoing metabolic inflammation.[7] By design, the relaxin-2 analogues discussed here cannot act at GR, a nuclear receptor, since they are optimized to harness the FcRn-γ mechanism and stay in circulation. Relaxin-2, in contrast, directly binds GR and travels to the nucleus.[13,14] Even upon direct *in-vitro* comparison with relaxin-2 in nuclear magnetic resonance binding, the B7 analogue does not bind GR.[15]

Apart from GR, relaxin-2 uses the Wnt1-β-catenin pathway to increase myocardial expression of the fast sodium channel (Nav1.5) and the tight-junction protein, connexin-43, an effect that underlies the protective effects towards atrial fibrillation as well as ventricular arrhythmia. This has recently been demonstrated in the ZSF1 rat model of HFpEF.[16] Of note, the single most frequent cause of cardio-vascular death in recent phase-III HFpEF trials is arrythmia,[17] which emphasizes the potential importance of this finding. Again, while details of the relaxin-2-Wnt1 pathway are still under examination, biased agonism at RXFP1 may miss this pivotal effect: Figure 1 shows that human relaxin-2, but not B7 increase Nav1.5 expression in ventricular cardiomyocytes freshly isolated from aged F-344 rats. The aged rat model, in turn, is another established animal model for HFpEF.

Naturally, we do not have access to the above-mentioned analogues under development by the different companies. Also, from what the press release imparted[1] we cannot judge which and if any of the arguments forwarded here are related to this

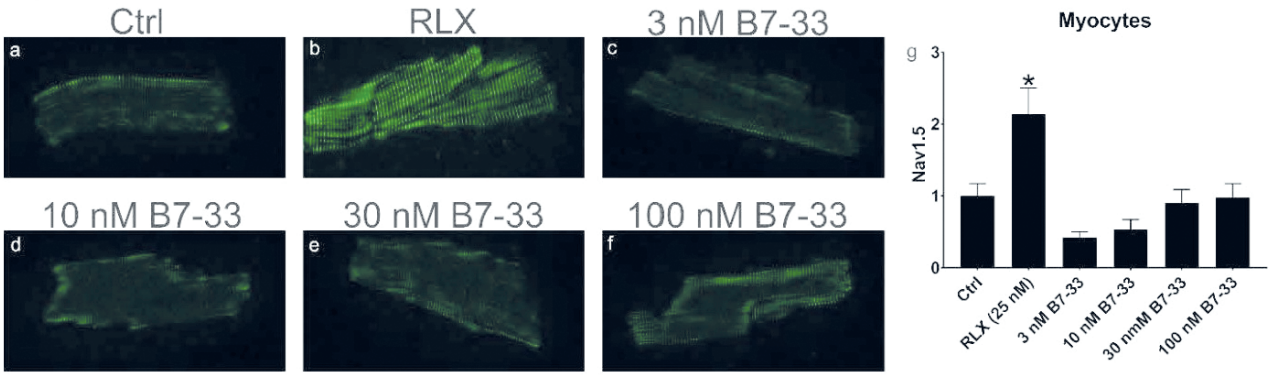

Figure 1. Relaxin-2, but not B7-33 increased Nav1.5 expression in isolated cardiomyocytes from aged F-344 rats. Relaxin (b) increased Nav1.5 expression compared to control cells (a) after 48-hour treatment. Treatment of cardiomyocytes with B(7-33) for 48 hours at increasing doses (c-f) had no effect on Nav1.5 expression compared to control. n ≥19 cells/group. Green, Nav1.5. 60x magnification; *p<0.05 compared to control.

study termination. Yet, we intend to make a case for the therapeutic use of full-length, native-structure human relaxin-2 in HF and especially in HFpEF. We need a fully pleiotropic drug for a most complex clinical syndrome.

How then to overcome the short half-life of human relaxin-2? We have demonstrated that it suffices to administer native (non-retarded) relaxin-2 once daily.[16,18] Long after the peptide has been cleared from circulation there is evident relaxin-2 binding to the myocardium,[18] which is based on another distinct feature, native relaxin's very slow off-rate from RXFP1. In RXFP1-overexpressing HEK cells,[19] this slow off-rate of native relaxin-2 is strikingly different from that of B7, a biased agonist.

Again, has eventually the time come to abandon hope of relaxin-2-derived therapies for HF? Not at all, at closer look and equipped with sufficient details of relaxin-2 pharmacology. More results using full-length human relaxin-2 in pre-clinical models of HFpEF as well as human HFpEF specimens to come, and a phase-II trial in immediate preparation.

Clinical summary box

Compared to native relaxin-2, the long-acting analogues described here contain sequence and structural modifications and are coupled to molecules approximately 2- to 5-fold larger than relaxin-2 itself. This prevents renal and hepatic clearance and thereby increases circulatory half-time. First, heightened immunogenicity and decreased therapy control may be an unwanted by-product of this peptide engineering. Second, the modifications change affinity and residence time of the analogues at the relaxin-2 receptor, as well as accessibility of intracellular compartments. This is called biased agonism and bears the consequence that essential signalling pathways activated by native relaxin-2 (e. g., glucocorticoid receptor, Wnt1 path) can no longer be exploited for HFpEF treatment.

Contributions

TBD drafted and conceptualized the manuscript; GS supervised the experimental work leading to Figure 1 and revised the manuscript. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest

TBD is CEO of Relaxera GmbH & Co. KG, a German company developing synthetic human relaxin-2 for heart failure and related indications. GS has received funding from Relaxera.

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