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EDITORIAL

The (apparent) sacubitril/valsartan sex interaction in heart failure with preserved ejection fraction: not the result of relaxin effects but of BNP action?!

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The results of the recently published PARAGON-HF trial with sacubitril/valsartan[1] have left the scientific community with another formally neutral large-scale trial in heart failure with preserved ejection fraction (HFpEF). The findings of the complementary trial, PARALLAX,[2] as presented in the late-breaking trial sessions of the virtual ESC Congress 2020,[3] have not really changed this perspective on HFpEF trials, either. Both trials have generated mixed results, at best, with PARAGON-HF narrowly missing its primary endpoint and PARALLAX reaching its primary biomarker-based endpoint, change in N terminal pro brain natriuretic peptide to Week 12, yet being neutral for the co-primary endpoint of the change in 6 min walk test distance to Week 24, and for both secondary endpoints related to quality of life and symptom burden.

What currently ignites scientific discussion are the observed significant interactions for both left ventricular ejection fraction and sex.[1] The former appears to be embraced, as it appears to make sense to many, and the latter is largely ignored as biological plausibility is questioned. Also, there is no sign for the sex interaction in the PARADIGM-HF trial (investigating the effect of sacubitril/valsartan vs. placebo in heart failure with reduced ejection fraction patients).[4] Maybe, the sex interaction in PARAGON-HF (at a *P* value for interaction of <0.006 for the primary endpoint) is a chance finding.

But now, there is some surprising news. In the discussion of PARALLAX after the presentation in the late-breaking trial session at the virtual ESC Congress 2020, it was mentioned that the result for the second primary endpoint of this study (i.e. the change in 6 min walk test distance) also showed a significant sex interaction in favour of women with HFpEF having a benefit from the use of sacubitril/valsartan vs. placebo.[3]

This comment sets out to explore whether there could be biological plausibility for a sex interaction in the effects of sacubitril/valsartan vs. placebo in HFpEF patients. We suggest two pathways for this. First, effects of endogenous relaxin may cause this sex difference. Second, it is conceivable that the actions of B-type natriuretic peptide (BNP) differ between atria and ventricles, which might explain the striking difference (in terms of presence and absence of the sex interaction) between the PARAGON-HF and PARADIGM-HF trials.

Endogenous relaxin-2

At first sight, there could be the following link: angiotensin type-1 receptor (AT1R) blockers (ARBs) have recently been shown to completely block the anti-fibrotic effects of therapeutic relaxin, both in rodent models of renal and cardiac fibrosis and in human cardiac fibroblasts.[5,6] This is attributable to a direct interaction at receptor level—between RXFP1, the cognate receptor for relaxin-2, the AT1R, and the AT2R—and it enables antagonists to each receptor blocking agonist effects at the other receptors.[5] In other words, ARBs have been shown in these models to completely nullify relaxin's anti-fibrotic signalling. Until now, it is inconclusive if and which other relaxin effects may be affected by use of ARBs.

This could be relevant for *therapeutic* relaxin. Heterogeneity of the relaxin effects in RELAX-AHF2 and other trials depending on the underlying therapy with ARBs in the patients included there are not known yet, but such analyses could easily be performed.

Considering the situation in the clinical studies with sacubitril/valsartan, one has to take into account *endogenous* relaxin-2. Circulating levels in men are ~300-fold to 500-fold lower than therapeutic levels.[7] In women, the ratio depends on menopausal status: during the menstrual cycle, relaxin levels may rise to approximately one-tenth of therapeutic relaxin levels, and this occurs approximately 10 days

after the plasma surge of the luteinizing hormone.[8] In post-menopausal women, however, levels are similar to those determined in men.[7] As a result, these facts may serve to construct a sex difference regarding relaxin-2 and a pharmacological link to the ARB component of the drug under review (i.e. of the valsartan part of sacubitril/valsartan).

At closer look, however, there are data that render this hypothesis rather unlikely. The mean age of patients in PARAGON-HF was 73 years. Most if not all women enrolled should have been post-menopausal, which effectively abolishes the said sex difference in endogenous relaxin levels. Moreover, the blockade of relaxin effects by valsartan would have been present in the active control arm as well—then, the sex interaction favouring women's outcome would rather rest in the sacubitril part of the drug to which we presently do not see any clue. Further, we also have to recognize the different design of control groups. PARAGON-HF encompassed one valsartan-treated control group while PARALLAX had three control strata of which only one was on ARBs. For angiotensin-converting enzyme inhibitors, the second PARALLAX stratum, it has been demonstrated that they do not interfere with relaxin effects.[9] Lastly, if there is no sex difference regarding circulating relaxin levels, could receptor interaction (RXFP1–AT1R–AT2R) itself be sex-dependent? At present, we have no data corroborating this option (TBD, personal communication). Altogether, these considerations suggest to us that we should dismiss the relaxin hypothesis.

Synthetic human relaxin-2, however, is currently being developed for HFpEF, and in spite of its unique pleiotropic signalling, it poses a promising candidate for chronic HFpEF therapy.[10] It will therefore be most interesting to explore in pre-clinical HFpEF models, for example, the ZSF1 rat, if and to what extent sacubitril/valsartan interferes with therapeutic relaxin effects.

Potential role of BNP in concert with androgens

A recent paper by Tsai and co-workers[11] may give rise to an alternative explanation. Those researchers focused on *atrial* BNP effects and referred to previous work in mice indicating tumour necrosis factor (TNF) to induce atrial fibrosis.[12] In their work, BNP significantly synergized with TNF and enhanced TNF-induced atrial fibrosis. Furthermore, one hallmark of fibrosis induction and collagen deposition, up-regulation of MMP-2, was also observed in primary human atrial fibroblasts following BNP challenge. Although these results need to be confirmed by other researchers, they raise the possibility that ventricular and atrial BNP effects on extracellular matrix may differ because in ventricles, BNP is well established to counteract fibrosis.[13–15]

Androgens play a pro-fibrotic role in many circumstances and seem to act in a dual-hit fashion in various contexts. In genetically engineered mice lacking the natriuretic peptide receptor-A (NPR-A aka GC-A), androgens act to produce sex-dependent cardiac fibrosis and hypertrophy,[16] with no such effect in the wild-type animals. Of note, down-regulation of NPR-A is a common finding in human heart failure,[17] which renders this model even more relevant. In male rats on chronic angiotensin infusion,[18] castration greatly reduces angiotensin-related hypertension, cardiac hypertrophy, and fibrosis, and this effect is reversed by testosterone replacement. Furthermore, relaxin-knockout mice show cardiac and renal fibrosis in males only while pulmonary fibrosis is markedly accelerated in males.[19] Again, there is no sex-dependent difference in organ fibrosis in the age-matched wild types in this study.

Against this background, we may hypothesize that in male HFpEF patients treated with sacubitril/valsartan, elevated BNP acts as second hit in concert with androgens to increase atrial fibrosis. This would potentially account for the difference between PARADIGM-HF vs. PARAGON in terms of the occurrence of a sex interaction. In heart failure with reduced ejection fraction, left atrial changes may be a mere 'bystander'-type of consequence along the course of disease progression, while in HFpEF, the left atrium takes a more central stage in the pathophysiology of disease progression.[20] The strong relation between elevated BNP and the incidence of atrial fibrillation (with atrial fibrosis a major precondition to it) is well established.[21] It is mainly regarded as association reflecting heart failure severity, but as we have elaborated here, it may be causal.

Still a chance finding?

It is still a serious possibility for the described sex interaction of sacubitril/valsartan vs. placebo in HFpEF, seen in one trial for sure and suggested for a second one, to be a mere chance finding, especially in view of the negative result of the study, which should not allow extrapolation of subgroup analyses. The use of pre-specified subgroups cannot answer all questions, if there are unknown confounders. Post-hoc subgroup analyses have questionable statistical value and can be hypothesis-generating at best. In PARAGON-HF,[22] women had lower estimated glomerular filtration rate and N terminal pro brain natriuretic peptide levels at baseline, they were older and more obese, and they had less presence of coronary artery disease. There were differences regarding region and race regarding women and men in this trial. Also, there were regional differences in the effectiveness of the trial with an effect in western Europe similar to that seen in women and in those patients receiving mineralocorticoid receptor antagonists. Further, in regard of the therapeutic effect, the sex

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difference mainly related to lesser hospitalizations—it did not translate into more pronounced improvements of functional class or KCCQ results in women.

Another plausible explanation could be the pharmacodynamic effect of sacubitril/valsartan as women have a smaller volume of distribution than men. A fixed dose regimen may result in higher effective dose in women. This can be relevant with regard to the blood pressure-lowering effect of the medicine.

We have not seen the full PARALLAX results yet, to judge in full the extent of validation of the interaction finding of PARAGON-HF in PARALLAX. Furthermore, as already mentioned, the control group in PARALLAX was different with only one of three strata on ARBs. Hence, we will not be able to settle this issue conclusively here.

In summary, we have attempted to explore two hypotheses—an ARB interaction with endogenous relaxin and an androgen-dependent pro-fibrotic *atrial* BNP effect—that could confer biological plausibility to the apparent sex–treatment interaction with sacubitril/valsartan in the PARAGON-HF trial. In the public discussion, a lot of weight is given to the observed ejection fraction-based interaction. The sex interaction is similarly strong, but it is largely ignored so far. Sex differences in ejection fraction are partly used to explain the PARAGON-HF findings. What if we would regard sacubitril/valsartan as the first successful therapy for female patients with HFpEF? Would that be bad for women with HFpEF? There are very many of them, and we think the answer to the question is 'no'. We simply feel that this issue deserves a closer and more scientific look. Whereas we feel that the relaxin hypothesis is untenable in this context, it may be worthwhile spending more experimental effort to investigate in detail the potentially different effects of BNP at the atrial as opposed to ventricular level.

Conflict of interest

T.B.D. is CEO of Relaxera, a company developing relaxin-2 for cardiovascular indications. S.D.A. reports receiving fees from Abbott, Bayer, Boehringer Ingelheim, Cardiac Dimension, Impulse Dynamics, Novartis, Occlutech, Servier, and Vifor Pharma and grant support from Abbott and Vifor Pharma (all unrelated to this paper).

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