News Release

## Title

Corticotropin releasing hormone receptor 2 antagonist, RQ-00490721, for the prevention of pressure overload-induced cardiac dysfunction

## Key Points

**・The treatment of cardiovascular diseases has improved but the prognosis for heart failure remains poor. Therefore, there is a need for new treatment options for the management of heart failure.**

**・We developed a novel, oral, small molecule antagonist of CRHR2, RQ-00490721, to investigate the inhibition of CRHR2 as a potential therapeutic approach for the treatment of heart failure.**

**・RQ-00490721 showed sufficient oral absorption in mice and protected them from pressure overload-induced cardiac dysfunction.**

**・RQ-00490721 may act as a starting point to facilitate the development of novel therapies for the treatment of chronic heart failure.**

## Summary

G protein-coupled receptors (GPCRs) regulate the pathological and physiological functions of the heart. GPCR antagonists are widely used in the treatment of chronic heart failure. Despite therapeutic advances in the treatments for cardiovascular diseases, heart failure is a major clinical health problem, with significant mortality and morbidity. Corticotropin releasing hormone receptor 2 (CRHR2) is highly expressed in cardiomyocytes, and cardiomyocyte-specific deletion of the genes encoding *CRHR2* suppresses pressure overload-induced cardiac dysfunction. This suggests that the negative modulation of CRHR2 may prevent the progression of heart failure. However, there are no systemic drugs against CRHR2.

We developed a novel, oral, small molecule antagonist of CRHR2, RQ-00490721, to investigate the inhibition of CRHR2 as a potential therapeutic approach for the treatment of heart failure. *In vitro*, RQ-00490721 decreased CRHR2 agonist-induced 3′, 5′-cyclic adenosine monophosphate (cAMP) production. *In vivo*, RQ-00490721 showed sufficient oral absorption and better distribution to peripheral organs than to the central nervous system. Oral administration of RQ-00490721 inhibited the CRHR2 agonist-induced phosphorylation of cAMP-response element binding in the heart, which regulates a transcription activator involved in heart failure. RQ-00490721 administration was not found to affect basal heart function in mice but protected them from pressure overload-induced cardiac dysfunction.

## Research Background

Heart failure is a critical disease caused by various factors leading to myocardial damage,

with a 4-year mortality rate of 60% in developed countries. In developing countries, the incidence of heart failure is expected to increase to a level similar to that of developed countries as socio-economic development progresses. Although the treatment of cardiovascular diseases, such as coronary artery disease and hypertension, has improved, the prognosis for heart failure remains poor. Therefore, there is a need for new treatment options for the management of heart failure. G protein-coupled receptors (GPCRs) are the largest superfamily of cell surface receptors; these proteins are involved in various transmembrane signaling systems and play a role in numerous physiological and pathological processes. The GPCR family includes seven highly-conserved transmembrane receptors, which are considered good targets for drug therapy. GPCRs in the heart are involved in the regulation of cardiac function in response to extracellular stimuli, such as catecholamines and angiotensin II, and play a role in cardiac dysfunction and fibrosis. Therefore, GPCR antagonists are commonly used to treat patients with chronic heart failure. Several GPCRs are expressed in the heart, but only ß-adrenergic and angiotensin II receptor blockers are clinically targeted for the long-term treatment of patients with chronic heart failure. Recently, angiotensin receptor–neprilysin inhibitors, which increase the activity of natriuretic peptides, in addition to functioning as angiotensin II receptor blockers, have been spread worldwide. Despite the availability of these effective treatments, mortality and hospitalization rates have remained high for over 10 years, suggesting that additional uncharacterized GPCRs may also be involved in mediating the pathophysiology of the disease.

A non-biased quantitative RT-PCR (qRT-PCR) analysis, which determined the gene copy numbers of 475 GPCRs in adult murine cardiomyocytes, revealed that corticotropin releasing hormone receptor 2 (CRHR2) was the 4th most abundantly expressed GPCR in cardiomyocytes. Western blot analysis indicated that CRHR2 is exclusively expressed in the heart and is undetectable in other tissues. The continuous infusion of urocortin 2 (Ucn2), an endogenous CRHR2 agonist, has been reported to reduce the left ventricular ejection fraction in mice, suggesting that constitutive CRHR2 activation may be responsible for cardiac dysfunction. The decline of cardiac function is suppressed in CRHR2-deficient mice as a result of Ucn2 loading and transverse aortic constriction (TAC). These findings suggest that negative modulators of CRHR2 could form a new oral treatment option for heart failure. In this study, we investigated the *in vitro* and *in vivo* pharmacological profiles of the CRHR2 antagonist, RQ-00490721, and found it to be a safe and promising oral treatment option for chronic heart failure.

## Research Results

*In vitro antagonistic activity*

The potency of CRHR2 antagonist was determined by blocking Ucn2-induced 3′,5′-cyclic adenosine monophosphate (cAMP) production in CHO-K1 cells stably expressing human CRHR2. We discovered RQ-00490721 as a CRHR2 antagonist by examining its ability to inhibit Ucn2-induced cAMP production in a screen of over 100,000 compounds. The antagonistic effect of RQ-00490721, determined based on the 90% effective concentration

($EC_{90}$) of human (45 nM) or murine (0.5 nM) Ucn2, was found to be a $pIC_{50}$ value of 7.09 ± 0.03 ($n$ = 6) in humans and 6.33 ± 0.07 ($n$ = 6) in mice. The concentration response curves of human Ucn2 were shifted rightward, with no change in the maximum response. Moreover, the Schild slope was calculated as 1.00, suggesting that RQ-00490721 competes with Ucn2 for binding to CRHR2. The pA2 value of RQ-00490721 was found to be 7.12 ± 0.02 ($n$ = 3) for human CRHR2. We also examined the antagonistic activity of RQ-00490721 in CHO-K1 cells stably expressing murine CRHR2. The pA2 value of RQ-00490721 for murine CRHR2 was found to be 6.61 ± 0.16 ($n$ = 3). The variation in pA2 showed that the potency of RQ-00490721 against human CRHR2 was approximately 3-fold higher than that against murine CRHR2.

*In vitro evaluation of safety*

To examine potential off-target binding and selectivity issues of RQ-00490721, the inhibitory activity of the radioactively labeled ligand against 80 targets, including GPCRs, ion channels, and transporters, was assessed. RQ-00490721 showed less than 50% inhibition at a concentration of 10 uM against most of the targets, except for the human M1 muscarinic acetylcholine receptor (60% inhibition) and human glucocorticoid receptor (70% inhibition). The cardiovascular and drug-drug interactions of RQ-00490721 were also assessed *in vitro*. No inhibition of human ether-a-go-go-related gene (hERG) potassium channels or human cardiac sodium channels (hNav1.5), which are associated with cardiac safety, was noted (half maximal inhibitory concentration [$IC_{50}$] > 10 uM). Although induction or inhibition of cytochrome P450 (CYP), a critical drug-metabolizing enzyme, has the potential to cause drug-drug interactions, RQ-00490721 did not significantly inhibit the levels of CYP isoforms 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 ($IC_{50}$ > 10 uM).

*Pharmacokinetic profile of RQ-00490721*

RQ-00490721 (1 mg/kg) was administered intravenously to mice. It exhibited moderate clearance (0.18 L/h/kg) and volume of distribution at steady state (0.73 L/kg). After administering the same dose orally, the maximum plasma concentration ($C_{max}$) of RQ-00490721 was found to be 0.816 ug/mL, and the moderate-terminal elimination half-life was recorded as 3.8 h. The bioavailability was close to 100%, suggesting that RQ-00490721 was well absorbed upon oral administration. Upon administration of multiple oral doses of RQ-00490721 (100 mg/kg), no changes in the plasma $C_{max}$ and area under the curve (AUC) were observed between day 1 and day 5; the unbound or free concentration of RQ-00490721 was approximately 4.3-5.3 uM. The brain-to-plasma ratio of RQ-00490721 concentration was found to be 0.038, suggesting that RQ-00490721 showed higher distribution to the peripheral organs than to the central nervous system.

*Inhibitory effect on Ucn2-induced phosphorylation*

The CRHR2-mediated phosphorylation of cAMP-response element binding (CREB) at Ser133 enhances the expression of genes associated with heart failure. Immunoblot analysis showed that Ucn2 induced the phosphorylation of CREB at Ser133 in the heart in a dose-dependent manner. Next, we examined the ability of RQ-00490721 to suppress CRHR2-mediated CREB phosphorylation *in vivo*. The Ucn2-induced phosphorylation of CREB in the heart was

suppressed by RQ-00490721 in a dose-dependent manner, suggesting that orally administered RQ-00490721 suppresses the Ucn2-induced activation of CRHR2 in the heart.

*Consecutive oral administration of RQ-00490721*

RQ-00490721 (100 mg/kg) was orally administered twice daily for 4 weeks. No significant differences were noted in the body weight and blood pressure of the mice treated with the vehicle or those treated with RQ-00490721. Next, we performed an electrocardiogram analysis. PR interval, the interval between the onset of the P wave and the onset of the QRS complex, represents the intra-atrial or atrioventricular conduction. A long QT interval due to prolonged ventricular repolarization is associated with polymorphic ventricular tachycardia known as *torsades de pointes*. Electrocardiogram analysis showed no significant differences in the heart rate, PR interval, or QT interval of mice between the control and RQ-00490721-treated groups. Echocardiography also revealed no significant differences in fractional shortening between the two groups. These findings suggest that the repetitive administration of RQ-00490721 does not affect the physiological cardiac function in normal mice.

*Effect on pressure overload-induced cardiac dysfunction*

We investigated the effect of RQ-00490721 treatment on the attenuation of cardiac hypertrophy progression in mice. Mice with pressure overload due to TAC are expected to develop cardiac hypertrophy within 1-2 weeks. Echocardiography was performed before and after performing TAC surgery, and left ventricular fractional shortening was measured. Oral treatment with RQ-00490721 was started one week after the sham procedure or TAC surgery. Macroscopically, TAC showed a strong tendency to cause cardiac hypertrophy, and treatment with RQ-00490721 was found to suppress TAC-induced cardiac hypertrophy. In addition, RQ-00490721 inhibited the TAC-induced increase in the left ventricular weight-to-tibia length ratio. qRT-PCR analysis showed that RQ-00490721 decreased the TAC-induced gene expression of brain natriuretic peptide, which is secreted by cardiomyocytes in response to pressure and volume overload. Although TAC decreased cardiac fractional shortening at three weeks after the surgery, RQ-00490721 was found to protect mice from further cardiac dysfunction at 3 and 5 weeks after TAC surgery. Sustained pressure overload causes adverse cardiac remodeling with hypertrophy and fibrosis, leading to heart failure. We evaluated cardiomyocyte size by measuring the cross-sectional area in hematoxylin and eosin-stained heart sections. Four-week treatment with RQ-00490721 significantly reduced the TAC-induced increase in cardiomyocyte size. To investigate the fibrotic changes induced by TAC, we performed Picrosirius red staining. Four-week treatment with RQ-00490721 significantly reduced TAC-induced cardiac fibrosis in mice. CRHR2 is coupled to the G-proteins, Gαs and Gβγ, resulting in the activation of respective cellular signaling pathways. Because CRHR2 activates Gαs-cAMP-dependent protein kinase A (PKA)-CREB and Gβγ-AKT signaling, we investigated the effect of RQ-00490721 treatment on the attenuation of TAC-induced phosphorylation of CREB at Ser133 and AKT at Ser473. Treatment with RQ-00490721 decreased TAC-induced CREB and AKT phosphorylation in the heart, suggesting that RQ-00490721 suppressed pressure-overload-induced Gαs/PKA/CREB, and Gβγ/AKT pathway

activity. These data indicate that RQ-00490721 has the potential to suppress heart failure induced by pressure overload.

## Research Summary and Future Perspective

We developed a novel orally-available CRHR2 antagonist, RQ-00490721, and demonstrated its ability to prevent cardiac dysfunction in mouse models. RQ-00490721 may act as a starting point to facilitate the development of novel therapies for the treatment of chronic heart failure. However, further studies are required to determine the mortality after long-term RQ-00490721 treatment and the physiological conditions of chronic heart failure for which RQ-00490721 treatment is suitable.

## Publication

Biomedicine & Pharmacotherapy. 2021 Dec 23;146:112566. (Online ahead of print.)
DOI: https://doi.org/10.1016/j.biopha.2021.112566

Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Bio_Pha_220120.pdf