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Review

Targeting Brain Aminopeptidase A: A New Strategy for the Treatment of Hypertension and Heart Failure

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ABSTRACT

The pathophysiology of heart failure (HF) and hypertension are thought to involve brain renin-angiotensin system (RAS) hyperactivity. Angiotensin III, a key effector peptide in the brain RAS, provides tonic stimulatory control over blood pressure (BP) in hypertensive rats. Aminopeptidase A (APA), the enzyme responsible for generating brain angiotensin III, constitutes a potential therapeutic target for hypertension treatment. We focus here on studies of RB150/firibastat, the first prodrug of the specific and selective APA inhibitor EC33 able to cross the blood-brain barrier. We consider its development from therapeutic target discovery to clinical trials of the prodrug. After oral administration, firibastat crosses the gastrointestinal and blood-brain barriers. On arrival in the brain, it is cleaved to generate EC33, which inhibits brain APA activity, lowering BP in various experimental models of hypertension. Firibastat was clinically and biologically well

RÉSUMÉ

La physiopathologie de l'insuffisance cardiaque (IC) et de l'hypertension artérielle impliquerait une hyperactivité du système rénine-angiotensine (SRA) cérébral. L'angiotensine III, un peptide effecteur clé du SRA cérébral, exerce un effet stimulateur tonique de la pression artérielle (PA) chez les rats hypertendus. L'aminopeptidase A (APA), l'enzyme responsable de la production d'angiotensine III dans le cerveau, constitue une cible thérapeutique potentielle pour le traitement de l'hypertension. Nous nous concentrons ici sur les études du RB150/firibastat, le premier promédicament de l'EC33, inhibiteur spécifique et sélectif de l'APA, capable de traverser la barrière hémato-encéphalique après administration par voie orale. Nous présentons son développement depuis la découverte de la cible thérapeutique jusqu'aux essais cliniques du promédicament. Après administration orale, le firibastat traverse les barrières gastro-intestinale et

Hypertension has been identified as an important risk factor in a number of diseases. It has been implicated in coronary heart disease, heart failure, stroke, and kidney dysfunction, for example.[1-3] Hypertension control remains poor worldwide, despite the availability of treatments. Furthermore, it is increasing in prevalence as the population ages and obesity rates rise.[4] With the application of the new blood pressure (BP) thresholds recommended in the 2017 American College of Cardiology/American Heart Association guidelines, a prevalence of 45.6% was reported for hypertension in individuals over the age of 20 years in the United States, with even higher values for non-Hispanic black adults.[5] African Americans have been reported to display more severe hypertension than their white counterparts, with higher rates of both morbidity and mortality.[6] Many effective antihypertensive drugs are currently available. They include systemic renin-angiotensin system (RAS) inhibitors, such as angiotensin II type 1 receptor (AT_1R) antagonists, angiotensin I–converting enzyme (ACE; EC 3.4.15.1) inhibitors, and direct renin inhibitors.[7,8] Black patients respond less well to systemic RAS blockers than white patients,[9] probably because their high BP is accompanied by a decrease in systemic RAS activity leading to low renin concentrations and by high plasma arginine vasopressin (AVP) levels.[10] Monotherapies are ineffective against hypertension in more than half of all cases, and there is considerable interindividual variability in responses to particular compounds for all of the chemical families used. Most patients with hypertension need to take at least 2 antihypertensive drugs, including a RAS blocker, a calcium channel blocker, or a diuretic, to get their BP below the 140/90 mm

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tolerated, even at high doses, in phase I trials conducted in healthy human subjects. It was then shown to decrease BP effectively in patients of various ethnic origins with hypertension in phase II trials. Brain RAS hyperactivity leads to excessive sympathetic activity, which can contribute to HF after myocardial infarction (MI). Chronic treatment with oral firibastat (4 or 8 weeks after MI) has been shown to normalize brain APA activity in mice. This effect is accompanied by a normalization of brain RAS and sympathetic activities, reducing cardiac fibrosis and hypertrophy and preventing cardiac dysfunction. Firibastat may therefore represent a novel therapeutic advance in the clinical management of patients with hypertension and potentially with HF after MI.

hémato-encéphalique. À son arrivée dans le cerveau, il est clivé pour générer l'EC33 qui inhibe l'activité de l'APA dans le cerveau, faisant baisser la PA dans divers modèles expérimentaux d'hypertension. Le firibastat est cliniquement et biologiquement bien toléré, même à fortes doses, dans des essais de phase I menés sur des sujets sains. Il a ensuite été démontré qu'il réduisait efficacement la PA chez des patients de diverses origines ethniques souffrant d'hypertension, lors d'essais de phase II. L'hyperactivité du SRA cérébral entraîne une activité sympathique excessive, qui peut contribuer à l'IC après un infarctus du myocarde (IM). Il a été démontré qu'un traitement chronique de firibastat par voie orale (4 ou 8 semaines après l'IM) normalise l'activité cérébrale de l'APA chez la souris. Cet effet s'accompagne d'une normalisation de l'activité cérébrale du SRA et de l'activité sympathique, réduisant la fibrose et l'hypertrophie cardiaques et prévenant la dysfonction cardiaque. Le firibastat pourrait donc représenter une nouvelle avancée thérapeutique dans la prise en charge clinique des patients souffrant d'hypertension et potentiellement d'IC après un IM.

Hg threshold.[11-13] Resistant hypertension, which is defined as BP remaining above 140/90 mm Hg despite the use of at least 3 antihypertensive drugs (including a diuretic) is observed in 15% of the hypertensive population.[14-17] The incidence of resistant hypertension is growing, which creates additional challenges for BP control in patients with hypertension. Many different pharmacologic and pathophysiologic factors underlie resistant hypertension, but medical inertia and a lack of adherence, whether to lifestyle changes or to medical interventions, also play an important role.[18] The risks of morbid cardiovascular disease events and death are greater in patients with resistant hypertension. We therefore urgently need new classes of antihypertensives, with novel targets and diverse modes of action, to improve overall BP control.

The Brain Renin-Angiotensin System

There is evidence for the existence of a functional RAS in the brain, playing an important role in controlling body fluid homeostasis and cardiovascular function.[19-21] All known components of the systemic RAS—the precursor, the enzymes, the angiotensin peptides and the receptors—are present in the brain.[20-26] Angiotensin II (AngII; Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) is produced by the sequential enzymatic cleavage of the precursor angiotensinogen (AGT). AGT is cleaved by an aspartyl protease, renin (EC 3.4.23.15), to generate the inactive decapeptide AngI (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu). The C-terminal His-Leu dipeptide of AngI is then cleaved by a membrane-bound zinc metalloprotease, ACE, to generate AngII. AngII is then converted to AngIII (Arg-Val-Tyr-Ile-His-Pro-Phe), which is then metabolized to generate AngIV (Val-Tyr-Ile-His-Pro-Phe) by 2 membrane-bound monozinc aminopeptidases identified as aminopeptidase A (APA; EC 3.4.11.7) and aminopeptidase N (APN; EC 3.4.11.2), respectively. AngII can also be metabolized by angiotensin-converting enzyme type 2 (ACE2; EC 3.4.17.23) to generate Ang(1-7), which binds with high affinity to the G protein-coupled receptor Mas. AngI can also be cleaved by neutral endopeptidase 24.11 (EC 3.4.24.11)[27] and by thimet oligopeptidase (EC 3.4.24.15) to generate Ang(1-7)[28] (Fig. 1). Among the effector peptides of the brain RAS, AngII and AngIII have similar affinities for the type 1 (AT1) and type 2 (AT2) AngII receptors.[22,29-31] Both peptides are involved in controlling BP and body fluid homoeostasis through interaction with AT_1Rs.[22,25,32] AngII/AngIII pressor responses involve an increase in sympathetic nerve activity, synaptic inhibition of the baroreflex in the nucleus of the tractus solitarius and an increase in AVP release into the bloodstream.[33]

Hyperactivity of the brain RAS has been implicated in the development and maintenance of hypertension, and its prevention by pharmacological or genetic blockade of the brain RAS is associated with profoundly beneficial hypertension outcomes.[34-40] Moreover, resistant hypertension and sympathetic hyperactivity have been linked to brain RAS overactivation.[41] Thus, novel drugs targeting the brain RAS may be useful for treating hypertension and/or diseases associated with elevated sympathetic outflow, such as heart failure (HF).

Identification of the *in vivo* Metabolic Pathways of Brain AngII and AngIII

The enzymes potentially able to hydrolyze AngII and AngIII include 2 membrane-bound zinc metallopeptidases,[42-44] APA and APN, which are considered particularly good candidates for this function. Purified APA hydrolyzes the N-terminal Asp of AngII to generate AngIII *in vitro*,[45] whereas purified APN hydrolyzes the N-terminal Arg of AngIII to generate AngIV.[46] Both APA and APN have been detected in rodent brains by means of immunocytochemistry,[47,48] in situ hybridization,[49] and specific enzyme assays.[50,51] They are present, in particular, in brain structures known to contain AT_1Rs and angiotensinergic nerve terminals,[24,26,52] which suggests that APA and APN may be components of the brain RAS.

Specific and selective APA and APN inhibitors were required for studies aiming to determine whether APA and APN were involved in the metabolism of brain angiotensins *in vivo*. APA inhibitors were developed by rational design, taking into account APA substrate specificity, APA exopeptidase activity, and the presence of Ca^{2+} and Zn^{2+} atoms in the APA active site.[53] Chauvel et al.[54] designed the first specific and selective APA inhibitor, EC33 [(S)-3-amino-4-mercaptobutyl sulfonic acid], in which the GluSH carboxyl side-chain was replaced by a sulfonate moiety (Fig. 2) to increase the

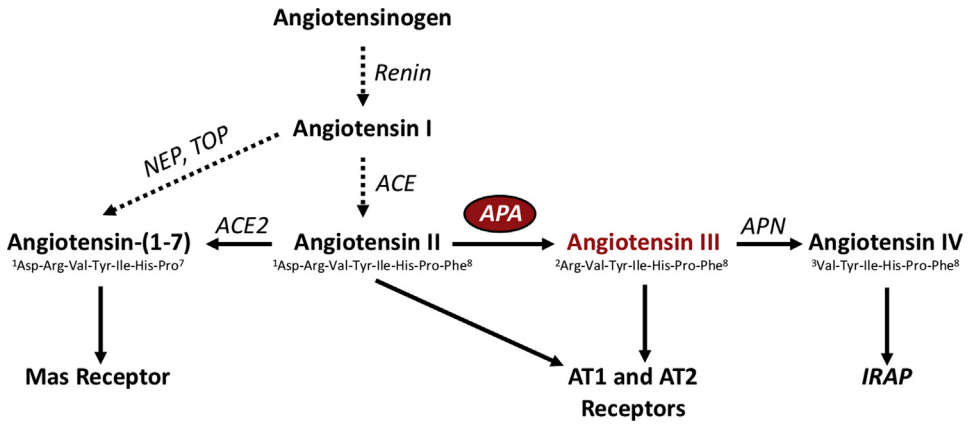

Figure 1. Schematic diagram of the brain renin-angiotensin system. ACE, angiotensin I–converting enzyme; ACE2, angiotensin-converting enzyme type 2; APA, aminopeptidase A; APN, aminopeptidase N; AT1 Receptor, angiotensin type 1 receptor; AT2 Receptor, angiotensin type 2 receptor; IRAP, insulin-regulated aminopeptidase; NEP, neutral endopeptidase; TOP, thimet oligopeptidase.

polarity of the side-chain and to strengthen the interaction with the calcium ion located in the S1 subsite of the APA active site, thereby increasing selectivity for APN. PC18 (2-amino-4-methylsulfonyl butane thiol) has also been developed as an effective APN inhibitor.[55] These inhibitors have been characterized pharmacologically with purified APA and APN. EC33 has been shown to inhibit APA (Ki = 0.29 µmol/L) almost 100 times more strongly than APN. PC18 inhibits APN (Ki = 0.008 µmol/L) 2150 times more strongly than APA (Ki = 17.2 µmol/L).[54-56]

The APA inhibitor EC33 (1-100 µg), injected into the cerebral ventricles (ICV) of conscious mice, has been shown to inhibit brain APA activity in a dose-dependent manner, with an IC_{50} of 12 µg.[57] Injections of a combination of EC33 and radiolabelled AngII into conscious mice totally prevent AngIII production in the hypothalamus.[58] Conversely, treatment with the APN inhibitor PC18 multiplies the half-life of AngIII by a factor of 4.[56] Thus, APA is clearly involved in the generation of brain AngIII from AngII *in vivo*, and APN in the metabolism of AngIV from AngIII.

Brain AngIII in Blood Pressure Control

AngIII was first put forward in 2003 as a more likely physiologically relevant brain RAS peptide for BP regulation than AngII.[59] This "AngIII hypothesis" has since been supported by the findings of numerous studies. AngII and AngIII display similar affinities for the AT_1Rs and AT_2Rs.[31] Harding et al.[60] showed that 93% of the angiotensin material released in the paraventricular nucleus after water deprivation or veratridine stimulation in a push-pull cannula study corresponded to AngIII. Only 6.8% of the angiotensins released were authentic AngII. AngII and AngIII have similar effects when administered centrally: They stimulate vasopressin release, decrease baroreceptor reflex function, and increase BP.[25,58,61-63]

Various studies have tried to determine the respective roles of AngII and AngIII in central BP control, through evaluations of the effects on BP following their ICV injection in hypertensive rats with and without APA or APN inhibitor treatment (EC33 or PC18, respectively). These studies have made use of 2 different experimental models of hypertension. The first, the spontaneously hypertensive rat (SHR), is a genetic model of hypertension that is sensitive to systemic RAS blockers. In the second model, the deoxycorticosterone acetate (DOCA)–salt rat, hypertension is salt and volume dependent but renin independent (low plasma renin concentrations), with resistance to systemic RAS blockers.

Central EC33 treatment blocks the pressor effect of ICV AngII in anaesthetized SHRs; the conversion of AngII into AngIII therefore seems to be required for an increase in BP.[36] ICV injection of EC33 alone immediately causes a total blockade of brain APA activity. This prevents AngIII formation in the brain and lowers BP in both hypertensive DOCA-salt rats and SHRs.[36,57] In contrast, intravenous treatment with a high dose of EC33 does not affect BP in hypertensive rats.[36] The ICV EC33–induced decrease in BP is therefore a central rather than systemic effect. In normotensive rats without brain APA or RAS hyperactivation, ICV EC33 treatment has no effect on BP.[57] We can therefore conclude that EC33 is an antihypertensive rather than a hypotensive agent.

These findings suggest that AngIII is one of the main effector peptides of the brain RAS. Further support for this conclusion is provided by the significant increase in BP observed after ICV injections of the APN inhibitor PC18 alone in SHR rats.[36] Prior treatment with losartan, which acts as an AT_1R antagonist, blocks this pressor response, whereas no such effect is observed with PD 123319, an AT_2R antagonist. By blocking, with PC18, the effects of APN on AngIII metabolism, it is thus possible to increase the levels of endogenous brain AngIII. This, in turn, leads to an increase in BP, through interactions between AngIII and AT_1Rs. Finally, EC33 completely prevents the PC18-induced increase in BP. This demonstrates the existence of the endogenous enzymatic cascade responsible for converting AngII by APA into AngIII, which is then used as the substrate of APN for AngIV generation.[36]

In accordance with these findings, other groups have reported that ICV infusions of APA significantly increase BP, whereas ICV infusions of APN decrease BP in SHRs, despite the high molecular masses of APA and APN (~ 120-130 kDa

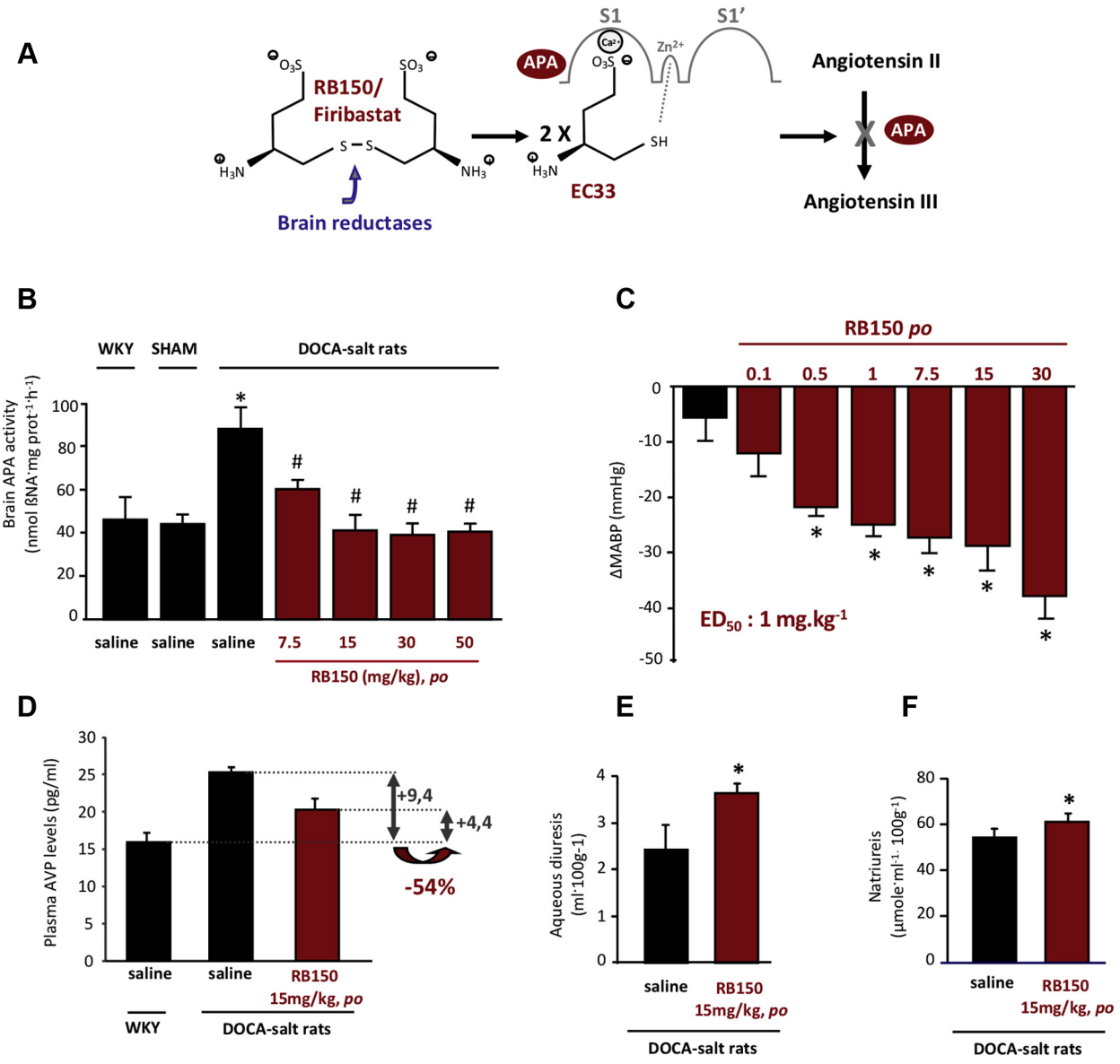

Figure 2. Effects of orally administered RB150/firibastat on brain APA activity, blood pressure, vasopressin release, diuresis, and natriuresis in alert DOCA-salt rats. (**A**) Conversion of angiotensin II into angiotensin III in the brain by the zinc metalloprotease APA. Structure of the APA inhibitor EC33 and its corresponding prodrug RB150/firibastat, consisting of 2 molecules of EC33 linked by a disulfide bridge. After oral administration, the bridge enables RB150/firibastat to cross the intestinal, hepatic, and blood-brain barriers and to penetrate the brain. In the brain, the disulfide bridge of RB150/firibastat is cleaved by reductases to release 2 active molecules of EC33, inhibiting brain APA activity and blocking the formation of brain angiotensin III. (**B**) Dose-response inhibition of brain APA activity 3.5 hours after the oral administration of RB150 (7.5-50 mg/kg) in conscious DOCA-salt rats. Mean ± SEM of 3-16 animals for each set of conditions. *$P < 0.05$ vs control values in Wistar Kyoto (WKY) and sham rats treated with saline solution. #$P < 0.05$ vs DOCA-salt rats treated with saline solution. (**C**) Mean arterial BP changes in alert DOCA-salt rats after oral RB150 administration. Peak changes in arterial BP (ΔMABP; mean ± SEM) after oral RB150 administration (0.1-30.0 mg/kg) in conscious DOCA-salt rats (n = 7 for each dose; **red bars**). *$P < 0.01$ vs changes in MBP values in DOCA-salt rats given saline solution orally (analysis of variance; **black bar**). Baseline MABP in DOCA-salt rats was 149.5 ± 3.5 mm Hg (n = 38). (**D-F**) Effects of the oral administration of RB150 on systemic vasopressin release, the urinary excretion of water and natriuresis in DOCA-salts rats. (**D**) Hypertensive DOCA-salt rats received either acute oral RB150 treatment (15 mg/kg orally; **red bar**) or saline solution (**black bars**), whereas normotensive WKY rats received only saline solution. Plasma AVP levels were determined 3 hours after treatment. The difference in plasma AVP levels between WKY and DOCA-salt rats receiving saline solution orally was 9.4 pg/mL, and the difference in plasma AVP levels between WKY and DOCA-salt rats receiving RB150 (15 mg/kg orally) was 4.4 pg/mL. The mean ± SEM of 6 animals analyzed individually is shown for each condition; unpaired Student *t* test: $P < 0.01$ vs values for WKY rats receiving saline solution. (**E, F**) After acclimation to metabolic cages over a period of 3 days, DOCA-salt rats received either acute oral RB150 treatment (15 mg/kg; **red bar**) or saline solution (**black bar**). They were then returned to the metabolic cages for the measurement of (**E**) urinary water excretion, and (**F**) natriuresis over a 5-hour period. Mean ± SEM of 4 animals analyzed individually, for each condition; paired Student *t* test: *$P < 0.05$ vs values for DOCA-salt rats receiving saline solution. APA, aminopeptidase A; AVP, arginine vasopressin; BP, blood pressure; DOCA, deoxycorticosterone acetate; MBP, mean blood pressure. Modified from Bodineau et al.[73] with permission from the American Heart Association.

for the monomer).[64] High brain AngIII levels probably underlie the pressor effect, whereas the BP decrease may reflect an increase in AngIII metabolism. Furthermore, a 59% smaller AngII-induced increase in BP has been reported after the ICV infusion of an antiserum blocking APA activity.[65] Finally, Wright et al.[59] investigated the effects of the metabolism-resistant analogues D-Asp[1]AngII and D-Arg[1]AngIII on blood pressure, following their ICV injection into conscious normotensive rats with and without EC33 and PC18 treatments. Their conclusion was that AngIII acted as a centrally active ligand of the brain RAS, controlling BP. Brain APA, which generates brain AngIII, is therefore a promising therapeutic target for the treatment of hypertension justifying the development of potent and selective APA inhibitors as central-acting antihypertensive agents.

However, it is well established that AngII and AngIII have the same affinity for AT_1Rs.[66] To explain why the blockade of AngIII formation by EC33 induces BP decrease, we hypothesize that AngII does not accumulate owing to the activation of other metabolic pathways. These enzymes may be dipeptidyl aminopeptidase III[67] converting AngII into Ang(3-8) (AngIV) which subsequently acts on IRAP[68] or ACE2 converting AngII into Ang(1-7), which binds with high affinity to the Mas receptor.[69]

In agreement with this hypothesis, Grobe et al.,[70] showed that in kidney sections incubated with exogenous AngII, after APA blockade by glutamate phosphonate, a specific APA inhibitor,[71] there was an increase in the conversion of exogenous AngII to Ang(1-7), resulting in an increase in Ang(1-7) levels. A similar pathway could also occur in the brain.

Firibastat, an Orally Active Aminopeptidase A Inhibitor Prodrug as a Novel Centrally Acting Antihypertensive Agent

APA inhibitors for use as central antihypertensive agents must cross the blood-brain barrier and inhibit brain APA activity after systemic administration. EC33 is unable to do this, which greatly limits its potential for clinical use. Prodrugs of thiol inhibitors of zinc metallopeptidases, such as neutral endopeptidase 24.11 or APN,[72] made through the formation of disulfide bridges to create dimers of the active compound, can considerably increase their central bioavailability. RB150 [4,4′-dithio bis(3-amino butyl sulfonic acid)] (Fig. 2), is a systemically active E33 prodrug developed specifically for this purpose. It is a dimer of 2 EC33 molecules linked by a disulfide bridge.[57]

Within the prodrug, the thiol group of RB150 is engaged in the disulfide bridge. It cannot, therefore, interact with the zinc atom present in the APA active site essential for its catalytic activity. However, the reduced form of RB150 obtained *in vitro* in the presence of dithiothreitol inhibits purified APA ($Ki = 0.20 \pm 0.02$ µmol/L) similarly to EC33. RB150 was subsequently renamed firibastat by the World Health Organisation.

When administered orally in hypertensive DOCA-salt rats or SHRs, RB150/firibastat crosses the intestinal, hepatic, and blood-brain barriers and enters the brain.[73,74] Once in the brain, the disulfide bridge of RB150/firibastat is immediately cleaved by brain reductases, generating 2 active molecules of EC33, which inhibit brain APA activity, block the formation of brain AngIII, and decrease BP and AVP release in conscious hypertensive rats without modifying heart rate (HR).

BP begins to decrease 2 hours after oral firibastat administration in hypertensive rats. This decrease is maximal between 5 and 9 hours after administration and persists for up to 15 hours (although it is no longer significant by that time). The effect of the drug is no longer detectable 24 hours after its administration (Fig. 2). No tolerance to the antihypertensive effect of RB150/firibastat was observed after chronic daily treatment for 24 days.[75] In contrast, oral RB150/firibastat, like EC33 administered by the ICV route, does not lower BP in normotensive rats; it therefore acts as an antihypertensive agent without effect when BP is normal.[73-75]

Studies in experimental models of hypertension have shown the antihypertensive effect of firibastat to be due to 3 different mechanisms: 1) a decrease in vasopressin release from the posterior pituitary into the bloodstream, increasing diuresis and reducing extracellular volume, 2) a decrease in sympathetic tone, decreasing vascular resistance, and 3) an improvement in baroreflex function (Fig. 3).[73-76] These observations provide insight into the role of brain AngIII in BP regulation in alert DOCA-salt rats and SHRs and led to the selection of RB150/firibastat as the lead candidate drug for clinical development.[74,77]

Clinical Trials of Firibastat in Hypertensive Patients

In a first-in-human phase Ia study (ClinicalTrials.gov Identifier: NCT01900171), the safety/tolerability, pharmacokinetics, and pharmacodynamic effects of single-ascending oral doses of firibastat were determined in humans.[78] Healthy male volunteers ($n = 56$) were randomly assigned to receive single oral doses of 10-1250 mg firibastat or placebo. All doses of firibastat were clinically and biologically well tolerated. Pharmacokinetic analysis demonstrated that exposure to firibastat was proportional to dose and suggested that once or twice daily oral dosing would be a suitable regimen for future studies. Firibastat did not significantly alter plasma renin or copeptin concentrations, plasma and urine free aldosterone, or cortisol concentrations. No significant change was observed in supine HR, systolic BP, or diastolic BP in any of the treatment groups.[74,78] In a second phase Ib clinical study (ClinicalTrials.gov Identifier: NCT01900184), the safety and tolerability of multiple oral doses of up to 750 mg twice daily for 7 days was confirmed in healthy subjects, but skin eruptions occurred with higher doses (1000 mg twice daily).[79] Multiple oral doses of firibastat up to 750 mg twice daily for 7 days had no effect on circulating renin-angiotensin-aldosterone levels, BP, or HR in healthy normotensive subjects on a regular sodium diet.

Following the success of phase I studies, the first investigation of the safety, tolerability, and BP effects of brain APA inhibition with the use of firibastat for 4 weeks was performed in patients with primary hypertension in a phase IIa proof-of concept crossover study (ClinicalTrials.gov Identifier: NCT02322450).[80] Firibastat treatment (250 mg twice daily for 1 week, with up-titration

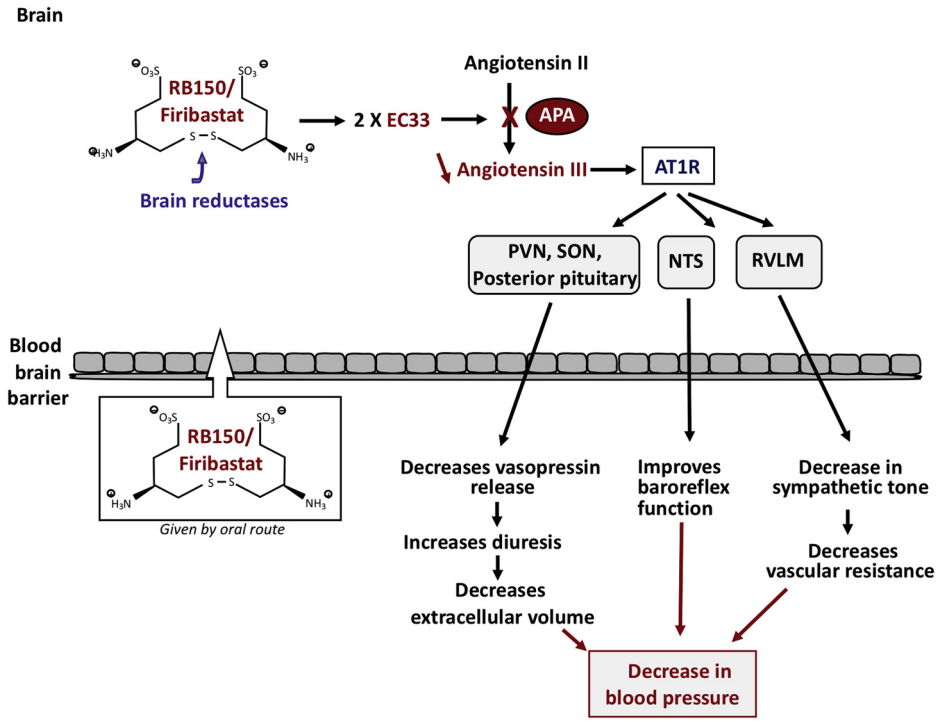

Figure 3. Mechanism of action of the APA inhibitor prodrug RB150/firibastat in the control of BP in hypertensive rats. Conversion of angiotensin II into angiotensin III in the brain by the zinc metalloprotease APA. Structure of the APA inhibitor EC33 and its corresponding prodrug RB150/firibastat, consisting of 2 molecules of EC33 linked by a disulfide bridge. After oral administration, the disulfide bridge enables RB150/firibastat to cross the blood-brain barrier and to penetrate the brain. In the brain, the disulfide bridge of RB150/firibastat is cleaved by reductases to release 2 active molecules of EC33, which inhibit brain APA activity. This results in a decrease in the formation of brain angiotensin III, which is known to have a tonic stimulatory effect on BP in hypertensive rats. This leads to a decrease in BP via 3 different mechanisms: 1) decreasing vasopressin release, 2) reducing sympathetic neuron activity, and 3) improving baroreflex function. APA, aminopeptidase A; AT1R, angiotensin type 1 receptor; BP, blood pressure; NTS, nucleus of the tractus solitarius; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla; SON, supraoptic nucleus. Modified from Marc et al.[74] with permission from the American Heart Association.

to 500 mg twice daily for 3 weeks) decreased daytime ambulatory systolic BP and office systolic BP by 2.7 and 4.7 mm Hg, respectively, vs placebo in the intention-to-treat population (34 patients). However, the difference between the groups was not statistically significant, owing to the small number of patients included. Interestingly, in the per-protocol population (29 patients), firibastat treatment induced a larger decrease in daytime ambulatory systolic BP (median [interquartile range (IQR)], −9.4 [−12.5 to −3.0] mm Hg) in patients with a basal value of daytime ambulatory systolic BP from 154 to 172 mm Hg, whereas placebo treatment had no effect (median [IQR], 0.75 [−5.5 to −1.9] mm Hg).[80] Thus the higher the basal daytime ambulatory systolic BP, the more marked was the firibastat-induced decrease in BP. These results are consistent with the antihypertensive effect of firibastat reported in experimental models of hypertension and with the absence of a BP decrease in healthy normotensive subjects treated with firibastat.[74,75,78] Moreover, firibastat did not affect safety parameters or systemic RAS activity in patients with primary hypertension. Firibastat was well tolerated in this study, with the exception of 1 episode of reversible skin allergy.

These results were subsequently used to guide the design of the phase IIb clinical trial, the NEW-HOPE trial (ClinicalTrials.gov Identifier: NCT03198793), in patients with hypertension and higher cardiovascular risks.[81] NEW-HOPE was an 8-week open-label phase IIb multicenter study performed in the United States to determine the efficacy and safety of firibastat in hypertensive patients of multiple ethnic origins. A total of 256 overweight or obese patients with hypertension, 54% of whom were black and Hispanic, received firibastat for 8 weeks (250 mg twice daily orally for 2 weeks, then 500 mg twice daily if automated office blood pressure (AOBP) was > 140/90 mm Hg; and hydrochlorothiazide 25 mg daily was added after 1 month if AOBP was ≥ 160/110 mm Hg). After 8 weeks of treatment, the changes in BP levels from baseline were highly significant, showing decreases of 9.5 mm Hg (95% confidence interval [CI] −10.7 to −7.3; $P < 0.0001$) for systolic AOBP and 4.2 mm Hg (95% CI −5.5 to −3.3) for diastolic AOBP ($P < 0.0001$).[81] Similar decreases in systolic AOBP were observed in African Americans (decrease of 10.5 mm Hg; $P < 0.0001$) and in non-African Americans (decrease of 8.9 mm Hg; $P < 0.0001$). Decreases in systolic and diastolic AOBP were also identical for the 215 subjects (85% of the subjects enrolled) who received firibastat as a monotherapy (ie, firibastat alone with no hydrochlorothiazide). The most frequent adverse events were headaches (4%) and skin reactions (3%). No angioedema was reported. No changes in blood concentrations of potassium, sodium, or creatinine were observed. No decrease was observed in hematocrit in patients with normal estimated glomerular filtration rate exposed to 8

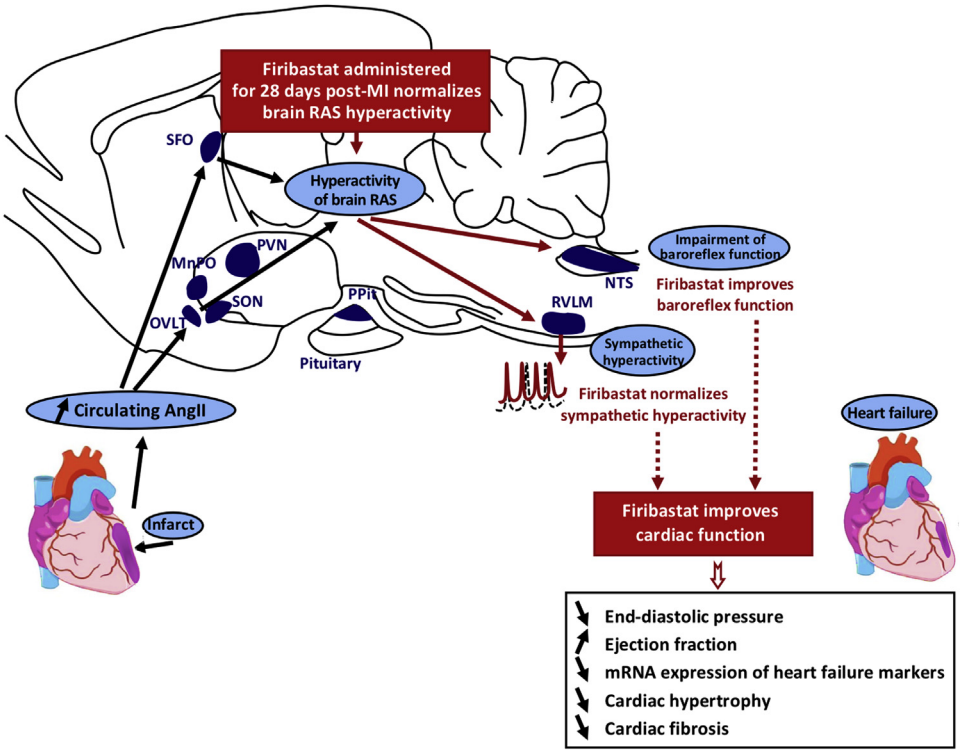

Figure 4. Schematic representation of the mode of action of RB150/firibastat in the prevention of cardiac dysfunction after myocardial infarction in rodents. After myocardial infarction (MI), ischemia induces a loss of cardiomyocytes and fibrous scars are formed. Circulating angiotensin II levels increase, penetrate the circumventricular organs (SFO and OVLT), and stimulate angiotensin type 1 receptors located in these structures. This induces brain RAS hyperactivity, leading to sympathetic hyperactivity[93] and an impairment of baroreflex function.[89] Sustained hyperactivity of the sympathetic nervous system has serious adverse consequences contributing to the progression of heart failure. Chronic treatment with RB150/firibastat for 4 or 6 weeks after MI in rats or mice normalizes brain RAS hyperactivity, leading to a normalization of sympathetic activity and improvements in baroreflex function.[76] RB150/firibastat improves cardiac function through several different effects: 1) left ventricle (LV) end-diastolic pressure reduction, 2) improvement of ejection fraction, 3) decrease in mRNA levels for various heart failure markers, including myosin heavy chain 7, B-type natriuretic peptide, and A-type natriuretic factor, 4) decrease in cardiac hypertrophy (LV end-systolic diameter and end-systolic volume), and 5) decrease in cardiac fibrosis (connective tissue growth factor and collagen I/collagen III).[90] MnPO median preoptic nucleus; NTS, nucleus of the tractus solitarius; OVLT, organum vasculosum of the lamina terminalis; PPit, posterior pituitary; PVN, paraventricular nucleus; RAS, renin-angiotensin system; RVLM, rostral ventrolateral medulla; SFO, subfornical organ; SON, supraoptic nucleus. Modified from Wescott et al.[89] with permission from NRC Research Press (© Canadian Science Publishing or its licensors) and from Boitard et al.[90] with permission from the Center for Interdisciplinary Research in Biology, Collège de France.

weeks of firibastat.[81] Despite the fact that firibastat has a central but not a systemic action, it will be important to evaluate if the longer-term exposure to firibastat as well as its administration to patients with chronic kidney disease coaffect hematopoiesis and lower hemoglobin levels as reported for ACE inhibitors/angiotensin receptor blockers.[82] Overall, the NEW-HOPE study provides strong evidence of the efficacy of firibastat for decreasing BP in a diverse high-risk population known to have a poor BP response to systemic renin-angiotensin system blockers, such as ACE inhibitors or AT_1R antagonists, owing to high salt sensitivity, low plasma renin activity, or sympathetic nervous system overactivity.[83] These data therefore provide support to further investigate the use of firibastat as an alternate therapy in patients with resistant hypertension.[83]

For this purpose, a key multicentre placebo-controlled phase III study, FRESH (**F**iribastat in Treatment-**Res**istant **H**ypertension; sponsored by Quantum Genomics),[84] will be conducted in subjects whose hypertension remains uncontrolled despite the use of at least 2 antihypertensive classes, including a diuretic, at the maximum tolerated doses.

Another Application of RB150/Firibastat in the Prevention of HF Following Myocardial Infarction

HF remains a major cause of morbidity and mortality. Myocardial infarction (MI)—induced ischemic cardiomyopathy is one of the main causes of the deleterious cardiac remodelling leading to HF. Interventional and pharmacologic therapies are available, but the search for new, more effective therapies remains important. Beta-blockers, antiplatelet drugs, statins, and ACE inhibitors are the standard treatments after acute MI. A number of new compounds for preventing MI-induced left ventricular (LV) remodelling and HF have emerged, including dual neprilysin-AT_1R blockers[85] and sodium-glucose cotransporter 2 inhibitors.[86,87] Targeting the brain RAS with the orally active APA inhibitor prodrug firibastat may also be an effective way of treating HF after MI and providing cardiovascular benefits. Indeed, circulating AngII increases after MI, and reaches circumventricular organs, such as the subfornical organ and the organum vasculosum of the lamina terminalis, in which AngII stimulates AT_1R and induces hyperactivity of the brain RAS.[88]

This brain RAS overactivity induces sympathetic hyperactivity and impairs baroreflex function, leading to deleterious LV remodelling and dysfunction[76,89,90] (Fig. 4). Sustained sympathetic nervous system hyperactivity has serious adverse consequences contributing to the progression of HF.

Various routes of firibastat administration have been evaluated in murine experimental models of HF induced by permanent ligature of the descending coronary artery. Chronic ICV administration of firibastat in rats after MI for 4 weeks normalizes brain APA hyperactivity and sympathetic hyperactivity, improves baroreflex function, and prevents cardiac dysfunction.[76] Chronic oral firibastat administration, initiated 2 days after ischemic injury and pursued for 28 or 56 days after MI in mice, improves cardiac function by increasing ejection fraction, decreasing LV end-diastolic pressure without modifying LV peak systolic pressure. Firibastat treatment also reduces the expression of various HF biomarkers even more effectively than enalapril treatment and attenuates both cardiac hypertrophy and fibrosis.[90] Chronic oral firibastat treatment for 4 weeks after MI in rats also improved cardiac contractility (dP/dt max) without lowering LV peak systolic pressure.[91] These results provided support for the design of an ongoing clinical phase II double-blind, active-controlled, dose-titrating efficacy and safety study (QUORUM; ClinicalTrials.gov Identifier: NCT03715998)[92] comparing the effects of firibastat with those of ramipril, a standard ACE inhibitor treatment, for preventing LV dysfunction in patients after acute MI.

Conclusion

Targeting the brain RAS with novel agents, such as the first-in-class APA inhibitor prodrug firibastat, has been shown to be effective against hypertension. After oral administration, firibastat crosses the blood-brain barrier, where it is then cleaved to generate EC33, which inhibits brain APA activity. This prevents the formation of brain AngIII, a key brain RAS effector peptide involved in BP control, leading to a normalization of BP in various experimental hypertension models, particularly those based on salt sensitivity.

Clinical Phase IIa and IIb trials have demonstrated the efficacy of brain APA blockade for lowering BP in patients with hypertension. This approach is particularly effective in black patients, who frequently display both salt sensitivity and poor responses to ACE inhibitor or AT_1R antagonist monotherapy. Confirmation of the safety and efficacy of firibastat for BP control in phase III trials could lead to the establishment of a new class of centrally acting antihypertensive agents, improving BP control in patients with difficult-to-treat or resistant hypertension.

Brain RAS hyperactivity leads to sympathetic hyperactivity and an impairment of baroreflex function. LV dysfunction, with harmful LV remodelling, may occur as a result. Long-term firibastat treatment after MI has been shown to restore normal brain APA activity in rodents, thereby normalizing brain RAS and sympathetic activities, preventing cardiac dysfunction and attenuating cardiac hypertrophy and fibrosis. Firibastat treatment thus has potential for use to prevent and treat HF after MI.

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