End-organ protection in hypertension by the novel and selective Rho-kinase inhibitor, SAR407899

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Abstract

AIM: To compare the therapeutic efficacy of SAR407899 with the current standard treatment for hypertension [an angiotensin converting enzyme (ACE)-inhibitor and a calcium channel blocker] and compare the frequency and severity of the hypertension-related end organ damage.

METHODS: Long-term pharmacological characterization of SAR407899 has been performed in two animal models of hypertension, of which one is sensitive to ACE-inhibition (LNAME) and the other is insensitive [deoxycorticosterone acetate (DOCA)]. SAR407899 efficiently lowered high blood pressure and significantly reduced late-stage end organ damage as indicated by improved heart, kidney and endothelial function and reduced heart and kidney fibrosis in both models of chronic hypertension.

RESULTS: Long term treatment with SAR407899 has been well tolerated and dose-dependently reduced elevated blood pressure in both models with no signs of tachyphylaxia. Blood pressure lowering effects and protective effects on hypertension related end organ damage of SAR407899 were superior to ramipril and amlodipine in the DOCA rat. Typical end-organ damage was significantly reduced in the SAR407899-treated animals. Chronic administration of SAR407899 significantly reduced albuminuria in both models. The beneficial effect of SAR407899 was associated with a reduction in leukocyte/macrophage tissue infiltration. The overall protective effect of SAR407899 was superior or comparable to that of ACE-inhibition or calcium channel blocking agents.

Conflict of interest: There is no conflict-of-interest to disclose.
channel blockade. Chronic application of SAR407899 protects against hypertension and hypertension-induced end organ damage, regardless of the pathophysiological mechanism of hypertension.

CONCLUSION: Rho-kinases-inhibition by the SAR407899 represents a new therapeutic option for the treatment of hypertension and its complications.

Key words: Hypertension; End organ damage; Rho-kinase; Angiotensin converting enzyme-inhibition

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Core tip: Rho-kinase (ROCK) is considered an important target in cardiovascular diseases, e.g., a hypertension and nephropathy. Currently available treatment only moderately alleviates the progression of chronic kidney diseases. We have identified a novel, potent and selective inhibitor of ROCK (SAR407899) and characterized its effects in vivo. The therapeutic efficacy of SAR407899 has been compared to the current standard treatment for hypertension in two animal models of hypertension, one of which is sensitive and the other insensitive (deoxycorticosterone acetate) to angiotensin converting enzyme-inhibition. ROCK-inhibition by the SAR407899 may represent a new therapeutic option either as a monotherapy or in combination with existing modern therapeutics for the treatment of hypertension and its complications.

INTRODUCTION

Hypertension increases the risk of target organ damage, including heart hypertrophy, heart ischemia, kidney dysfunction or failure, cerebrovascular events and malfunction of the endothelial tissue[1-5]. The contractile response of smooth muscle tissue is controlled at different levels by a tight balance of pro-contractile and relaxing mechanisms. In particular, it has been persuasively shown that Rho-kinases (ROCK1 and ROCK2) are intimately involved in the transmission of contractile signaling within smooth muscle tissue[6-13]. Upon activation of the small GTPase RhoA by ligand-bound specific GPCRs, ROCKs, the downstream effectors of RhoA, phosphorylate the myosin light chain phosphatase and the myosin regulatory light-chain itself, resulting in a net increase in activated myosin. This promotes smooth muscle contraction and actin cytoskeleton re-organization. Inhibition of ROCKs leads to relaxation of vascular smooth muscle cells and, consequently, to a decrease in blood pressure[14-20]. Several inhibitors of ROCK (in particular, fasudil and Y27632) have been extensively used to evaluate the role of ROCK in cardiovascular physiology and pathology. In addition, fasudil is used in Japan to treat cerebral vasospasm after focal cerebral ischemia or aneurysmal subarachnoid hemorrhage. However, both inhibitors have a moderate specificity, moderate potency and short duration of action in vivo that limit their clinical use. Therefore, the development of a more potent and specific inhibitor with a better pharmacokinetic profile is needed to explore the potential of ROCK inhibition in the therapy of hypertension and its complications. We have identified a novel ROCK-inhibitor, SAR407899, and previously characterized its acute effects in vitro and in vivo[21]. Here, we describe the long-term effects of SAR407899 treatment in two animal models of hypertension, one being sensitive (Nω-Nitro-L-arginine methyl ester hydrochloride, LNAME) and the other being insensitive [deoxycorticosterone acetate (DOCA)] to angiotensin converting enzyme (ACE)-inhibition. The DOCA-induced hypertension model is characterized by a hypervolemic and low plasma renin status, which promotes resistance to ACE-inhibition, whereas the LNAME model is normovolemic and displays a high renin activity in plasma[22]. The results of large clinical trials (IDNT, RENAAL and IRMA-2) revealed that the current standard treatment only modestly (approximately 20%) reduces the progression of chronic kidney diseases. From these data it can be concluded that a simple decrease of blood pressure is not sufficient for kidney protection. Our results indicate that chronic inhibition of the ROCK kinases efficiently controls blood pressure and significantly reduces the frequency and severity of the hypertension-related end organ damage.

MATERIALS AND METHODS

DOCA and LNAME-induced hypertension

Adult male Sprague Dawley rats (190 to 210 g, Harlan Winkelmann, Borchheim, Germany), were treated with DOCA salt or Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) to induce hypertension. To compare the pharmacological potency of ROCK-inhibition with the current anti-hypertension drugs, the individual blood pressure lowering effect of the respective compounds was measured in spontaneous hypertensive rats (SHR). Oral application of SAR407899 at 3 mg/kg lowered blood pressure in conscious telemetred SHR by 26 ± 4 mmHg (n = 10), which was comparable to the action of amlodipine at 3 mg/kg (blood pressure reduction by 33 ± 8 mmHg, n = 10) and ramipril at 1 mg/kg (blood pressure reduction by 21 ± 7 mmHg, n = 10). Therefore, the animals were divided into the following groups: (1) Control; (2) DOCA or LNAME; (3) DOCA or LNAME + SAR407899 at 3 mg/kg; (4) DOCA or LNAME + SAR407899 at 10 mg/kg;
kg; (5) DOCA or LNAME + ramipril at 1 mg/kg and (6) DOCA or LNAME + amlopidine at 3 mg/kg. All DOCA-salt treated animals underwent a unilateral nephrectomy, received a subcutaneous injection of DOCA (30 mg/kg; Sigma Chemical, St. Louis, MO, United States) dissolved in sesame oil once a week and 1% NaCl in the drinking water ad libitum. All LNAME groups received 40 mg/kg per day LNAME in the drinking water ad libitum. After a 4-wk treatment, the animals were placed into metabolic cages and 24 h-urine samples and blood samples were taken to analyze kidney function. Urinary and plasma creatinine levels were determined using standard kits (Roche diagnostics, Basel, Switzerland) on a Hitachi 912 E analyzer (Hitachi, Mountain View, Calif., United States). Urinary albumin was measured using a standard kit from Progen (Mikroflural, Progen, Heidelberg, Germany) and was normalized to urinary creatinine concentrations. After 5 wk of treatment, measurements of: (1) blood pressure (invasively, two hours after the last treatment); (2) organ weights; and (3) endothelial function were taken. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication no. 85-23, revised 1996).

Invasive blood pressure measurement
The animals were anaesthetized with an intraperitoneal injection of thiopental (0.1 g/mL per 100 g body weight). The common carotid artery was catheterized with a heparinized microcatheter (diameter 1.6 mm, Vycon, France). Blood pressure was measured with a Hugo Sachs hemodynamic system (March-Hugstetten, Germany) using the software Hemodyn.

In vitro vascular and heart function
Assessment of in vitro vascular function was performed as described earlier[21,25,26]. Heart function was determined using a Langendorff-setup in the working heart perfusion mode. This technique allows the heart to perform its physiological pumping action, i.e., it performs pressure/volume work. The working heart technique therefore provides a complete analysis of heart function. Heart power is an integrative parameter and is calculated by formula (1):

\[ HW \ (J) = 133.3 \ \frac{[N/m^2]}{mmHg} \times (ALP_{\text{mean}}-PL_{\text{mean}}) \ \text{mmHg} \times SV (m^3) \]

\[ + 0.5 \times 1.004 \ \text{(kg/m^3)} \times SV (m^3) \times \{SV (m^3)/[\pi \times r^2 (m^3)] \times ET (s)\}^2 \]

where HW-heart power, ALP-mean-afterload pressure, PL-mean-preload pressure, SV-stroke volume, and ET-Ejection time. If not otherwise indicated, chemicals were obtained from Sigma (Deisenhofen, Germany).

RT PCR
Real-time quantitative PCR was performed using the QuantiTect Probe RT-PCR Kit (Qiagen, Hilden, Germany). Each sample was assayed in quadruplicate. For relative quantification of gene expression, the ΔCt method was used with GAPDH as a control. Amplification of the target and housekeeping genes was detected simultaneously using differently fluorescent-labeled Taq Man probes (Col1A1: Rn00801665_g1, CD3: Rn01417941_g1 and CD68: Rn01495634_g1), which were obtained from Applera/Applied Biosystems (Foster City, United States). Amplification linearity of the target and housekeeping genes within the multiplex RT-PCR was assessed by performing RT-PCR reactions with dilutions of the templates. RT-PCR reactions and data acquisition was performed using the iCycler-iQ-Thermocycler (Bio-Rad Laboratories GmbH, Munich, Germany). Relative gene expression was calculated as fold induction vs control samples.

Histology
Heart and kidneys underwent a standard fixation procedure and standard haematoxylin-eosin and sirius red staining. The hearts and kidneys were analyzed with regard to incidence and extent of fibrosis, inflammatory events, glomerulosclerosis and tubular atrophy. A semi-quantitative score was assigned to each specimen by an experienced pathologist ranging from 1 (minimal changes) to 5 (marked alterations) at a standard magnification of 4 to 20-fold. All histopathological analyses were performed in a blinded fashion. Anti-podocin staining was performed using anti-podocin antibodies (Sigma-Aldrich, United States).

Statistical analysis
All values are given as the mean and standard error of mean. Normality of the distribution and the homogeneity of variance were checked using the Levene test. For group comparisons, one-way analysis of variance (ANOVA) or two-way ANOVA was performed followed by Dunnett’s post-hoc test using the SAS version 8.2 software. Differences between groups were considered significant if \( P < 0.05 \); \( n \) represents the number of specimens or animals tested.

The statistical methods of this study were reviewed by and complies to the standard of Sanofi-Aventis GmbH Deutschland.

RESULTS

Effect of SAR407899 on body weight, blood pressure and kidney function
The long-term effects of SAR407899 in DOCA- and LNAME-induced hypertension were compared to those of the current standard anti-hypertensive drugs, namely ramipril (ACE-inhibitor) and amlopidine (calcium channel blocker, CCB). Figure 1 depicts the effects of SAR407899 on body weight of the DOCA- and LNAME-hypertensive animals. Treatment with SAR407899 was well tolerated and showed a significant protective effect on body weight in both hypertensive animal models (Figure 1A and C). Factors involved in the continuous body weight loss are not known and most likely depend on hypertension related end-organ damage, including proteinuria. Ramipril showed protective effects
The lack of efficacy of amlodipine has been linked to the reduce albuminuria in the DOCA or LNAME models. Dose administered, amlodipine did not significantly LNAME model but not in the DOCA model. At the Ramipril significantly reduced albuminuria in only the by the measurement of albuminuria. In both models, able to control blood pressure efficiently in both models doses in the LNAME rats. Thus, only SAR407899 showed superior blood pressure lowering effects at both comparison to am lodipine and ramipril, SAR407899 all treatments significantly lowered blood pressure. In hypertensive model is characterized by hypervolemia and (Figure 2A) and LNAME-treated rats (Figure 2B). SAR407899 effectively reduced blood pressure in both hypertensive models. Because the DOCA-induced hypertensive model is characterized by hypervolemia and resistance to ACE-inhibition, it was not surprising that ramipril had no significant effect on blood pressure. At the dose employed, amlodipine non-significantly lowered blood pressure in the DOCA rats. In the LNAME rats, all treatments significantly lowered blood pressure. In comparison to amlodipine and ramipril, SAR407899 showed superior blood pressure lowering effects at both doses in the LNAME rats. Thus, only SAR407899 was able to control blood pressure efficiently in both models and was therefore superior to the reference substances.

Kidney function (Figure 2C and D) was assessed on body weight only in the LNAME model (Figure 1D), whereas amlodipine significantly protected the DOCA hypertensive animals from body weight loss (Figure 1B).

Figure 2 demonstrates the effects of SAR407899 (3 mg/kg and 10 mg/kg), ramipril (1 mg/kg) and amlodipine (3 mg/kg) on blood pressure in the DOCA- (Figure 2A) and LNAME-treated rats (Figure 2B). SAR407899 significantly reduced blood pressure in both hypertensive models. The inability of CCB’s to dilate renal efferent vessels, which is critical for improvement of the renal microcirculation.

Long term treatment effects of SAR407899 on heart and endothelial function

Figure 3 illustrates the effect of SAR407899 and of reference substances on heart function (A and B), measured in isolated Langendorff perfused hearts in the working heart mode and on vessel function in vitro (C and D). Hearts of hypertensive the DOCA rats and the LNAME rats were functionally compromised. At an afterload pressure of 40 mmHg (basal afterload), a similar heart performance was found in all groups; however, at higher afterload pressures (60 and 80 mmHg), a significant reduction was found in heart function from the DOCA- and LNAME-treated rats. Long-term treatment with SAR407899 restored heart function in both groups. In contrast, treatment with either amlodipine or ramipril had no significant effect. Endothelial function was found to be severely compromised in the DOCA rats. Figure 3C and D show the effect of SAR407899 and of the reference substances on endothelial function. Long-term treatment with SAR407899 improved endothelial function in a dose-dependent manner (Figure 3C). As expected, ramipril had no effect on endothelial function of arteries from the DOCA-induced hypertensive rats (Figure 3D). Amlodipine had similar protective effects on endothelial function in the DOCA hypertensive rats.
(Figure 3D). The endothelial function of arteries of the LNAME-treated rats has not been assessed because LNAME exerts a potent and long-lasting inhibition of the endothelial nitric oxide synthase.

**Effect of SAR407899 on DOCA- and LNAME-induced heart and renal pathology**

Histological examination of the hearts of the DOCA- (Figure 4A-F) and LNAME-treated (Figure 4G-L) rats revealed a prominent perivascular fibrosis, massive infiltration of leukocytes into the interstitium and sclerotic changes. Figure 4A-C and 4G-I shows sirius red staining of DOCA- (Figure 4B) and LNAME- (Figure 4H) induced heart fibrosis in comparison to control (Figure 4A and G) and SAR407899 treatment (Figure 4C and I). Figure 4D-F and 4J-L shows haematoxylin eosin staining of DOCA- (Figure 4E) and LNAME- (Figure 4K) induced heart fibrosis in comparison to control (Figure 4D and J) and SAR407899 treatment (Figure 4F and L). In the hearts of the DOCA and LNAME rats, SAR407899 treatment at 10 mg/kg abolished the development of fibrosis (protection with SAR407899 at 3 mg/kg was less pronounced and data not shown). Chronic treatment of the LNAME hypertensive rats with SAR407899 attenuated leukocyte infiltration and perivascular fibrosis. Figure 4M and N summarize heart lesions according to the scoring procedure. The heart lesions of the DOCA rats were significantly reduced by SAR407899 at 10 mg/kg. Both reference compounds showed only marginal non-significant effects on heart lesions (Figure 4M). SAR407899 (at 3 and 10 mg/kg) and ramipril significantly diminished heart lesions in the LNAME-treated rats (Figure 4N). Histological analysis of kidneys from the DOCA- (Figure 5A-F) and LNAME- (Figure 5G-L) treated rats revealed severe lesions of glomeruli and tubuli. Figure 5A-C shows
the vascular wall, hyperplasia and degeneration of tubuli, and tubulointerstitial changes with inflammatory cell infiltration. Again, chronic treatment with SAR407899 attenuated these pathophysiological changes (Figure 5I).

Figure 5K shows the fading of podocin staining upon LNAME treatment indicating that podocin positive cells are damaged. Figure 5J demonstrates podocin staining in control animals. Long-term treatment with SAR407899 attenuated glomerular, vascular, and interstitial changes (Figure 5L). Figure 5M and N summarize kidney lesions according to the scoring procedure. The kidney lesions of the DOCA rats were significantly reduced by SAR407899 at 3 and 10 mg/kg. Both reference compounds showed only marginal non-significant effects on kidney lesions (Figure 5M). SAR407899 (at 3 and 10 mg/kg) and ramipril significantly diminished kidney lesions in the LNAME-treated rats (Figure 5N). Expression of genes characteristic of fibrosis and leukocyte infiltration of the kidneys of the DOCA- and LNAME-treated rats was measured using RT PCR (Figure 6A-F). The expression of collagen was significantly increased upon DOCA and LNAME.
Figure 4  Histological examination of the effect of SAR407899 on the heart. Sirius red staining of hearts of deoxycorticosterone acetate (DOCA) (A-C) and Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) rats (G-I) showed a strong induction of myocardial fibrosis. Upon treatment with SAR407899 at 10 mg/kg, significant protective effects were observed. Haematoxylin eosin staining of hearts of DOCA (D-F) and LNAME rats (J-L) showed perivascular fibrosis, massive infiltration of leukocytes into the interstitium and sclerotic changes. SAR407899 treatment at 10 mg/kg attenuated multifocal fibrosis, perivascular fibrosis and leukocyte infiltration. M and N: Summary of heart lesions and the effect of SAR407899 and reference substances in DOCA (M) and LNAME rats (N). A, D, G, J: Control; B, E; DOCA; H, K: LNAME; C, F, I, L: SAR407899 10 mg/kg.
Figure 5 Histological examination of the effect of SAR407899 on the kidney. A-C: Haematoxylin eosin staining of normal glomeruli in control rats (left), sclerotic changes, dilation and hypertrophy of glomeruli of deoxycorticosterone acetate (DOCA) rats (center) and protective effects of SAR407899 at 10 mg/kg (right); D-F: Podocin staining in kidneys. Upon DOCA treatment, massive loss of podocytes can be detected. SAR407899 at 10 mg/kg exerts a protective effect in the kidneys of DOCA rats and rescues podocytes; G-I: Haematoxylin eosin staining of normal glomeruli in control rats (left), severe fibrotic changes, infiltration of leukocytes, and hypertrophy of glomeruli of Nω-Nitro-L-arginine methyl ester hydrochloride (LNAME) rats (center), protective effects of SAR407899 at 10 mg/kg (right); J-L: Loss of podocytes upon LNAME treatment. SAR407899 at 10 mg/kg protected against loss of podocytes. Summary of kidney lesions and effect of SAR407899 and reference substances in DOCA (M) and LNAME rats (N). A, D, G, J: Control; B, E: DOCA; H, K: LNAME; C, F, I, L: SAR407899 10 mg/kg.
DISCUSSION

It has been generally accepted that RhoA-associated kinases (ROCK1 and ROCK2) have important functions in treatment. SAR407899 at 10 mg/kg significantly reduced collagen expression in the kidneys of the DOCA rats and non-significantly in the kidneys of the LNAME rats (Figure 6A and B). Leukocyte infiltration into kidney tissue was quantified by measuring CD3 (T lymphocytes) and CD68 (macrophages) mRNA abundance in the kidneys of the DOCA and LNAME rats. Upon DOCA or LNAME treatment, both parameters were significantly induced, thus indicating significant leukocyte infiltration. SAR407899 at 10 mg/kg efficiently reduced leukocyte infiltration in the kidneys of the DOCA rats and non-significantly in the kidneys of the LNAME rats (Figure 6E and F), presumably by inhibition of leukocyte migration.
not only in vascular smooth muscle contractility but also in actin cytoskeleton rearrangement, cell adhesion, cytokinesis and motility in various cell types\textsuperscript{[1,12,27,31]}. Two commercially available ROCK-inhibitors, fasudil and Y27632 were used in the majority of the published \textit{in vitro} and \textit{in vivo} studies. Both compounds were identified in the mid-1980s and were used to elucidate the pathophysiological role of ROCKs in several pathologies. Y27632 was first claimed in a patent in 1988\textsuperscript{[12,33]} as a calcium channel blocker, and fasudil was patented in 1986\textsuperscript{[34,35]}, also as a novel calcium channel blocker. Later, discovery of ROCKs\textsuperscript{[36]} led to the identification of these kinases as a direct target of both compounds. However, fasudil and Y27632 possess some unfavorable properties, including limited selectivity and importantly, a short duration of action. The latter might be a reason why fasudil was often administered at high doses, \textit{e.g.}, 30 mg/kg per day or 100 mg/kg per day\textsuperscript{[37,38]} \textit{in vivo} animal studies, which increases the probability of non-specific and toxic effects in different organs. In long-term animal studies of hypertension in which fasudil was used at low doses, \textit{e.g.}, 10 mg/kg, no significant blood pressure lowering effect and only a partial renoprotective effect were found\textsuperscript{[38,39]}.

SAR407899 is a selective and potent inhibitor of ROCK that has been extensively characterized \textit{in vitro}\textsuperscript{[21]}. Acute treatment of conscious hypertensive animals (SHR, SHR-sp, LNAME or DOCA-salt treated rats) with SAR407899 resulted in a strong and sustained fall in blood pressure\textsuperscript{[21]}. To evaluate the long-term effects of SAR407899, the compound was tested in two models of hypertension, one non-sensitive (DOCA) and the other sensitive (LNAME) to ACE-inhibition. The treatment effects of SAR407899 were compared to two current standard medications for high blood pressure, namely the inhibition of ACE (ramipril) or calcium channels blockade (amlodipine). As expected, treatment with ramipril demonstrated potent blood pressure lowering and end organ protective effects in the LNAME but not in the DOCA hypertension model\textsuperscript{[24,40,41]}. Because the DOCA hypertensive model is characterized by hypervolemia and low plasma renin levels, this model is insensitive to ACE-inhibition\textsuperscript{[23]}. The treatment effects of amlodipine at the given dose were small and non-significant in both models. Our data are in good agreement with several other studies in which amlodipine was found to be ineffective in protecting kidney function and structure in the DOCA rat or less effective than ACE-inhibitors in the LNAME rat\textsuperscript{[22,42,43]}. Even when amlodipine was dosed three times higher, \textit{e.g.}, at 10 mg/kg, no reduction in proteinuria or glomerular damage was found in the DOCA rats, although amlodipine efficiently lowered blood pressure. However, in other models, \textit{e.g.}, in the LNAME rats, treatment with amlodipine showed pronounced protective effects on end organ damage and myocardial protection combined with the efficient blood pressure lowering effect in different rat strains. The lack of efficacy of amlodipine has been further linked to the inability of CCBs to dilate renal efferent vessels to improve renal microcirculation\textsuperscript{[22,43-47]}. Long-term treatment with SAR407899 showed an efficient dose-dependent blood pressure reduction in both models with no signs of tachyphylaxia over the course of 35 d. Blood pressure lowering effects and protective effects on hypertension related end organ damage of SAR407899 were superior to ramipril and amlodipine in the DOCA rat. Typical end-organ damage observed upon chronic LNAME administration (hypertrophy and fibrosis of the heart, fibrosis of the kidney, glomerulosclerosis, tubular degeneration and leukocyte-infiltration) was significantly reduced in the SAR407899-treated animals as supported by histopathological and gene expression analyses. Albuminuria, which is considered the key parameter of kidney damage in general and of glomerular damage in particular, were strongly induced in the DOCA- and LNAME-challenged rats. Chronic administration of SAR407899 significantly reduced albuminuria in both models. Histologically visible improvements should translate into functional benefits (endothelial function and heart contractility) observed upon SAR407899 administration. Indeed, hearts of hypertensive the DOCA or LNAME animals treated with SAR407899 showed a significantly improved heart function (measured as heart power \textit{in vitro}). Moreover, endothelial-dependent relaxation \textit{in vivo} was significantly and dose-dependently improved after long-term treatment with SAR407899. Histological scoring and quantitative real time PCR analysis revealed a significant reduction in interstitial fibrosis, as measured by sirius red staining and collagen expression, respectively. SAR407899 treatment significantly reduced tissue expression of CD3 and CD68 (markers of infiltrating leukocytes and macrophages) in both models, which can be explained by the role of ROCKs in cellular migration and cytokinesis through modulation of the cytoskeleton. Similar data were reported by several groups studying the effect of ROCK inhibition on macrophage migration in atherosclerotic plaques\textsuperscript{[48-50]}. Therefore, the beneficial effects of SAR407899 do not depend on only blood pressure control but also the suppression of inflammatory and fibrotic events in the target organs. These data suggest that inhibition of ROCKs could bring clear therapeutic benefit through different mechanisms and is not limited to vasorelaxation only. Recently, other authors stated that potential benefits of ROCK-inhibition, direct or indirect, could be more far-reaching than first thought\textsuperscript{[13]}. Further studies using highly selective, potent, and safe ROCK inhibitors could open new perspectives for ROCK-based therapies in several different clinical indications.

**COMMENTS**

**Background**

Large clinical trials have demonstrated that current treatment with angiotensin converting enzyme-inhibitors, Angiotensinreceptor-blockers or calcium channel blockers only modestly reduces the progression of chronic kidney diseases. Hypertension increases the risk of target organ damage, including heart hypertrophy, heart ischemia, kidney dysfunction or failure, cerebrovascular...
lok minor. End organ protection by rho-kinase inhibition

Research frontiers
Rho-kinase (ROCK) is considered an important target for a variety of cardiovascular diseases, including hypertension and hypertension-related end-organ damage. Several inhibitors of ROCK have been extensively used to evaluate the role of ROCK in cardiovascular physiology and pathology. However, these inhibitors have a moderate specificity, moderate potency and short duration of action in vivo that limit their clinical use. Therefore, the development of a more potent and selective inhibitor with a better pharmacokinetic profile is needed to explore the potential of ROCK inhibition in the therapy of hypertension and its complications.

Innovations and breakthroughs
The authors have identified a novel, potent and selective inhibitor of ROCK (SAR407899) and characterized its long-term effects in two animal models of hypertension.

Applications
ROCK inhibition by the SAR407899 represents a new therapeutic option for the treatment of hypertension and its complications.

Terminology
Rho-kinases (ROCK1 and ROCK2) are intimately involved in the transmission of contractile signaling within smooth muscle tissue. Upon activation of the small GTPase RhoA by ligand-bound specific GPCRs, ROCKs, the downstream effectors of RhoA, phosphorylate the myosin light chain phosphatase and the myosin regulatory light-chain itself, resulting in a net increase in activated myosin. This promotes smooth muscle contraction and actin cytoskeleton re-organization. Inhibition of ROCKs leads to relaxation of vascular smooth muscle cells and, consequently, to a decrease in blood pressure.

Peer review
This article is particularly meaningful for hypertension-related end-organ damage.

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