BAY 41-2272 Treatment Improves Acetylcholine-Induced Aortic Relaxation in L-NAME Hypertensive Rats

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Abstract

Hypertension, an emerging problem of recent era, and many pathophysiological factors are participating to produce the disease. Nitric oxide (NO) is an important constituent to ameliorate hypertensive condition. Inhibition of endogenous NO synthase by L-N^G-Nitroarginine methyl ester (L-NAME) was responsible for generating hypertension in rats. BAY 41-2272 (5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1H-pyrazolo[3,4-b]pyridine-3-yl]-pyrimidin-4-ylamine), a soluble guanylyl cyclase activator, restricts rise of blood pressure and shows cardioprotective activity. The aim of the present study was to analyze effect of short-term BAY 41-2272 treatment on blood pressure and vascular function.

Male Wistar rats were randomly divided into three groups such as control (group-A), hypertensive (group-B), and BAY 41-2272-treated hypertensive (group-C) rats. Normal saline was administered intramuscularly to control rats for last 3 days (days 40, 41, and 42) of total 42 days treatment, whereas rats of group-B and group-C were treated with L-NAME hydrochloride in drinking water at 50 mg/kg body weight daily for 42 days. Also, normal saline and BAY 41-2272 were administered for last 3 days at two different dosages at 1 and 3 mg/kg body weight/day intramuscularly to group-B and group-C rats, respectively.

Administration of BAY 41-2272 for 3 days was not sufficient enough to decrease mean arterial pressure of hypertensive rats significantly. BAY at both the treatment dosages significantly ameliorate acetylcholine-induced maximal aortic relaxation compared with BAY-untreated hypertensive rats.

Findings of the present study indicate that even shorter period of BAY 41-2272 treatment (3 days) improves vascular relaxation.

Keywords

- hypertension
- L-NAME
- mean arterial pressure
- BAY 41-2272
- thoracic aorta
- improves acetylcholine relaxation
- Wistar rats

Hypertension is one of the most common emerging chronic diseases of developed and developing countries. There is a correlation between hypertension and cardiovascular diseases. Delayed in treatment proceed to stroke, congestive heart

failure, chronic kidney disease, and premature death.^{1,2} Several pathophysiological factors are participating in pathogenesis of hypertension.³ Nitric oxide (NO) is a signaling molecule of biological system and produces vascular relaxation through

activation of soluble guanylyl cyclase resulting into release of cGMP.⁴ In vivo NO biosynthesis has catalyzed by enzyme NO synthase.⁵ There are several NO synthase inhibitors in which L-N^G-Nitroarginine methyl ester (L-NAME) is an agent producing hypertensive model in laboratory animal such as rats.⁶ L-NAME is a strong inhibitor of both constitutive and neuronal NO synthase enzymes modulating NO level and influencing vascular function.⁷ BAY 41-2272 (5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1H-pyrazolo[3,4-b]pyridine-3-yl]-pyrimidin-4-yl-amine) is reported as the first product that stimulates soluble guanylyl cyclase not mediated through NO pathway, inhibit vascular smooth muscle growth and platelets aggregation, and possess cardioprotective action.^{8,9} BAY 41-2272 improves vascular function¹⁰ and a report from our laboratory reveals that BAY improves acetylcholine (ACh)-induced aortic relaxation in deoxycorticosterone acetate (DOCA) salt hypertensive rats.¹¹ BAY 41-2272 restricts the cardiovascular anomaly and improves vascular activity to neurotransmitters in L-NAME hypertensive rats.^{12,13} The aim of the present study was to analyze the effect of short-term BAY 41-2272 treatment on blood pressure and vascular function. Keeping the curative role of BAY 41-2272 in hypertension, the present study was planned to administer BAY intramuscularly once daily for 3 days.

Materials and Methods

Experimental Animals

Hypertension was induced in healthy male Wistar rats weighing 150 to 200 g and experimentation has been performed as per guidelines of Institutional Animal Ethics Committee. After environmental acclimatization rats were randomly divided into three groups, namely, control (group-A), L-NAME-hypertensive (group-B), and BAY 41-2272-treated hypertensive (group-C) Wistar rats. In control (group-A) rats, normal saline was administered intramuscularly for last 3 days, that is, days 40, 41, and 42, whereas group-B rats was treated with L-NAME hydrochloride (Cayman Chemical, Ann Arbor, MI) after dissolving in drinking water at 50 mg/kg body weight/daily for 42 days¹⁴ and normal saline has been administered for last 3 days. Rats of group-C was treated similar to group-B, and in addition, BAY 41-2272 has been administered intramuscularly for last 3 days (40, 41, and 42) at two different dosages at 1 and 3 mg/kg body weight/day after dissolving in normal saline.¹⁵

Mean Arterial Pressure Measurement

After the completion of 6 weeks, rats were anesthetized with pentobarbital sodium at 60 mg/kg, intraperitoneally to measure mean arterial pressure (MAP) by invasive method. Carotid artery of anesthetized rats was cannulized and connected to transducer attached to data acquisition system display on desktop Laboratory-Chart v6.1.3 (Powerlab, AD Instrument Australia, NSW, Australia).

Pharmacodynamics Study

Thoracic aorta from all experimental rats was isolated for organ bath study.¹⁶ Aortic rings of 3 to 4 mm size were mounted between two stainless steel wire hooks of 37-gauge under resting tension of 1.5 g in a thermostatically

controlled ($37 \pm 0.1^\circ\text{C}$) organ bath (Ugo-Basile, Varese, Italy) of 10-mL capacity containing modified Krebs–Henseleit solution (MKHS) continuously bubbled with medical gas ($74\%\text{N}_2 + 21\%\text{O}_2 + 5\%\text{CO}_2$). After acquiring equilibration, aortic rings were contracted with high potassium chloride (80 mM) depolarizing solution to check tissue viability. After accomplishment of maximum contraction plateau of high potassium chloride, aortic tissue was washed with MKHS. Once baseline was achieved, arterial rings again contracted to phenylephrine (PE) and further dose–response curve elicited with ACh, sodium nitroprusside (SNP) to analyze the changes of vascular function in BAY 41-2272-treated group.

Statistical Analysis

Vascular contraction and relaxation values, viz., maximal response (E_{max}) and concentration producing 50% of maximal response ($\text{EC}_{50}/\text{pD}_2$) were analyzed by nonlinear regression analysis to obtained sigmoid dose response curve with variable slope using Graph Pad Prism version 4.00 (San Diego, CA). Data were analyzed by Student–Newman–Keuls method for multiple group analysis and concentration-dependent dose response data by two-way analysis of variance followed by Bonferroni post hoc test. Differences in values were considered statistically significant at $p < 0.05$.¹⁷

Results

Effect of 3 Days BAY 41-2272 Treatment on Mean Arterial Pressure of L-NAME Hypertensive Rats

►Fig. 1 shows a significant increase of MAP that was recorded in L-NAME hypertensive rats compared with control rats. No significant changes in MAP were found in BAY-treated rats in comparison to BAY-untreated hypertensive rats.

Effects of 3 Days BAY 41-2272 Treatment on Phenylephrine-Induced Contraction in Rat's Aorta

An α -adrenoceptor agonist PE (1 nM–10 μM) added cumulatively with an increment of 0.5 log unit to study concentration-

Fig. 1 Effects of 3 days BAY 41-2272 treatment on mean arterial pressure of L-NAME hypertensive rats. ^{a,b}At $p < 0.05$, different superscripts vary significantly from each other, BAY-treated and BAY-untreated hypertensive rats^b vary significantly from control.^a L-NAME, L-N^G-Nitroarginine methyl ester.

Fig. 2 Effect of BAY treatment on dose dependent (A) contractile response and (B) maximal response (E_{max}) to PE on aortic rings from L-NAME hypertensive rats. *At $p < 0.05$, control rats vary significantly from L-NAME hypertensive rats. ^aSignificantly differ from control rats. ^bSignificantly differs from BAY-untreated hypertensive rats. L-NAME, L-N^G-Nitroarginine methyl ester; PE, phenylephrine.

dependent contraction of aortic rings isolated from different groups of rats (►Fig. 2A, B). A significant increase of maximal contraction response was found in aorta from L-NAME hypertensive rats ($E_{max} = 1.05 \pm 0.02$ g; $n = 9$) in comparison to control ($E_{max} = 0.90 \pm 0.03$ g; $n = 9$). BAY treatment at 3 mg/kg was significantly decreased maximal contractile response (►Table 1) to PE in aortic rings from BAY-treated rats ($E_{max} = 0.87 \pm 0.05$ g; $n = 5$) as compared with BAY-untreated hypertensive rats ($E_{max} = 1.05 \pm 0.02$ g; $n = 9$). Similarly, BAY at 1 mg/kg markedly decreased maximal contractile response to PE in BAY-treated rats as compared with BAY-untreated hypertensive rats. However, BAY at both treatment dosages had not shown any significant change of PE-induced potency as compared with BAY-untreated L-NAME hypertensive rats.

Effects of 3 Days BAY 41-2272 Treatment on Vasodilation Elicited to Acetylcholine in Phenylephrine Precontracted Aorta from L-NAME Hypertensive Rats
ACh (1 nM–10 μ M) added cumulatively with an increment of 0.5 log unit to observe effect of BAY on ACh-induced relaxation in PE precontracted aortic rings from BAY-

treated hypertensive rats (►Fig. 3A, B). A significant decrease ($p < 0.05$) of maximal relaxation response (►Table 1) to ACh was found in aortic rings from hypertensive rats ($E_{max} 39.33 \pm 1.33\%$; $n = 8$) in comparison to control ($E_{max} 104.9 \pm 1.07\%$; $n = 8$). However, a significant increase of ACh-induced ($p < 0.05$) maximal aortic relaxation was found in BAY-treated rats ($E_{max} = 51.99 \pm 2.64\%$; $n = 7$ and $58.63 \pm 4.0\%$; $n = 7$) at 1 and 3 mg/kg, respectively, in comparison to BAY-untreated hypertensive rats ($E_{max} 39.33 \pm 1.33\%$; $n = 8$).

Effects of 3 Days BAY 41-2272 Treatment on Vasodilation Elicited to Sodium Nitroprusside on Phenylephrine Precontracted Aorta from L-NAME Hypertensive Rats

SNP (0.1 nM–10 μ M) added cumulatively with an increment of 0.5 log unit to observe effect of BAY on SNP-induced relaxation on PE precontracted aortic rings isolated from rats of different group (►Fig. 4A, B). A significant increase ($p < 0.05$) of dilatory potency (►Table 1) of SNP was found on aortic rings from hypertensive rats ($pD_2 8.46 \pm 0.05$; $n = 6$) compared with control ($pD_2 7.67 \pm 0.05$; $n = 5$). Three days

Table 1 Depicting effects of 3 days BAY 41-2272 treatment on maximal response (E_{max}) and potency (pEC_{50}/pD_2) of ACh, SNP, and PE

Groups	PE		ACh		SNP	
	pEC_{50}/pD_2	E_{max} (%)	pEC_{50}/pD_2	E_{max} (%)	pEC_{50}/pD_2	E_{max} (%)
Control	6.69 ± 0.08 ($n = 9$)	0.90 ± 0.03 ($n = 9$)	7.64 ± 0.04 ($n = 8$)	104.9 ± 1.07 ($n = 8$)	7.64 ± 0.05 ($n = 5$)	105.6 ± 1.65 ($n = 5$)
L-NAME	6.95 ± 0.06 ($n = 9$)	1.05 ± 0.02^a ($n = 9$)	7.62 ± 0.13 ($n = 8$)	39.33 ± 1.33^a ($n = 8$)	8.46 ± 0.05^a ($n = 6$)	104.8 ± 1.21 ($n = 6$)
L-NAME + BAY 41-2272 (1 mg/kg)	6.64 ± 0.11 ($n = 6$)	0.96 ± 0.04 ($n = 6$)	6.97 ± 0.16^b ($n = 7$)	51.99 ± 2.64^b ($n = 7$)	8.52 ± 0.07^a ($n = 5$)	106.8 ± 1.67 ($n = 5$)
L-NAME + BAY 41-2272 (3 mg/kg)	6.74 ± 0.17 ($n = 5$)	0.87 ± 0.05^b ($n = 5$)	7.42 ± 0.27 ($n = 7$)	58.63 ± 4.00^b ($n = 7$)	8.23 ± 0.05^a ($n = 5$)	103.6 ± 1.35 ($n = 5$)

Abbreviations: ACh, acetylcholine; L-NAME, L-N^G-Nitroarginine methyl ester; PE, phenylephrine; SNP, sodium nitroprusside.

^aSignificantly vary from control rats, at $p < 0.05$.

^bSignificantly vary from L-NAME hypertensive rats, at $p < 0.05$.

Fig. 3 Effect of BAY treatment on dose dependent (A) vasodilation and (B) maximal relaxing response (E_{max}) to ACh on aorta from L-NAME hypertensive rats. *At $p < 0.05$, control rats vary significantly from hypertensive rats. [†]At $p < 0.05$, BAY-treated hypertensive rats (3 mg/kg) vary significantly from BAY-untreated hypertensive rats. [‡]At $p < 0.05$, L-NAME hypertensive rats vary significantly from control rats. ^bBAY-treated vary significantly from BAY-untreated L-NAME hypertensive rat. Ach, acetylcholine; L-NAME, L-N^G-Nitroarginine methyl ester.

BAY treatment did not show significant changes in vasodilatory potency (pD_2) and maximal relaxation response (E_{max}).

Discussion

The aim of the present study was to establish the role of hypertension in vascular dysfunction. Physiologically, conduit vessel or resistance vessel shows similar response to hypertension as far as vascular dysfunction is concerned. Structural changes induced by hypertension in both type of vessels also appear identical.^{18,19} In the present study, modulation on vascular smooth muscle functions by NO deficiency was studied. There are several similar studies which have implicated hypertension in endothelial dysfunction of both conduit and resistance vessels.^{20,21} In this study, after intramuscular administration of BAY 41-2272 (1 and 3 mg/kg) daily for 3 days was not sufficient enough to

produce significant decrease of MAP of hypertensive rats. Zanolini et al¹² reported chronically administration of BAY 41-2272 improves vascular function and shows capability to inhibit development of hypertension in rats. In the present study, BAY 41-2272 was unable to restrict the development of MAP of rats because of variation in dose, frequency, and routes of administration or period of treatment. However, improvement of vascular function was noted in BAY-treated rats as compared with BAY-untreated hypertensive rats indicating participation of BAY to improve vascular function.

In the present study, a significant increase of PE elicited maximal contraction was found in hypertensive rats compared with control. However, BAY at 3 mg/kg significantly decreases PE-induced maximal contraction in hypertensive rats indicating contractile action has been promoted by BAY. The increase of PE-induced contraction was found in hypertensive rats because of less availability of NO in L-NAME

Fig. 4 Effect of BAY treatment (A) on relaxation response and (B) on maximal response produced by SNP on PE precontracted aorta from hypertensive rats. *At $p < 0.05$, control rats vary significantly from hypertensive rats. [†]At $p < 0.05$, BAY-treated hypertensive rats vary significantly from BAY-untreated hypertensive rats. L-NAME, L-N^G-Nitroarginine methyl ester; PE, phenylephrine; SNP, sodium nitroprusside.

hypertensive model.²² NO synthase inhibitor increases PE-induced contraction of rats aorta incubated with L-NAME and shifted concentration-dependent contraction to left.^{23,24} On the contrary, L-NAME shifted the curve of BAY 41-2272-induced relaxation of basilar artery to right pretreated with L-NAME.¹⁰ Therefore, an increase of phenylephrine-induced contraction may decrease basal NO level in L-NAME hypertensive rats.^{25,26} Also, inhibition of NOS in L-NAME model helps release endothelium-derived contracting factors producing vasocontraction of rat's aorta.²⁷

BAY at two different dosages produces significant increase of Ach-induced maximal aortic relaxation found in BAY-treated compared with BAY-untreated hypertensive rats. BAY 41-2272 is a NO independent soluble guanylyl cyclase agonist; ameliorate relaxation of aorta isolated from rabbit through synergistic activation of soluble guanylyl cyclase (sGC) by both BAY and NO.²⁸ Ach-induced relaxation of mesenteric artery of spontaneous hypertensive rats was enhanced in presence of BAY 41-2272 by combating oxidative stress, improves cGMP dependent and independent relaxation, increasing NO bioavailability and sGC expression.²⁹ Aorta isolated from DOCA hypertensive rats incubated with BAY 41-2272 improves Ach elicited relaxation.¹¹ Therefore, in the present study, BAY 41-2272 improves Ach-induced aortic relaxation by reducing oxidative stress and/or activating sGC.^{11,29}

L-NAME produces hypertensive rats by decreasing vascular NO level. SNP, a NO donor was compensating the effect of L-NAME by providing NO and producing SNP-induced maximal relaxation similar to control group. Similar SNP-induced maximal relaxation was found on aorta from BAY-treated hypertensive rats compared with BAY-untreated hypertensive rats. In NO deficient aorta of L-NAME hypertensive, SNP-induced relaxation was found at par with control rats indicating sensitization of SNP effect in hypertensive rats.²⁶ Although a significant increase in potency was found in BAY-treated and -untreated L-NAME hypertensive rats compared with control because of increase of sensitivity to SNP mediated through sGC or NO.^{30,31}

Hypertension produces morphological and chemical changes in blood vessels (conduit and resistance vessels) which result in variations in their response to physiological mediators of blood pressure.³² Changes such as arterial stiffness, vessel wall thickness, and wall stress induced by hypertension are considered as factors responsible for such variations.^{33,34} Besides, increase in elastin and collagen contents of blood vessel is implicated in the development of stroke in hypertensive patients.³² The vascular functional disparity so induced leads to inhibition of ACh-induced relaxation of blood vessels isolated from genetic and experimental hypertensive rats.³⁵⁻³⁷ In the present study, sGC activator BAY 41-2272 has demonstrated ameliorating effect on such vascular functional disparity, thereby restoring some normal response of blood vessel from hypertensive models. Therefore, the result of the present study discloses that even 3 days BAY 41-2272 treatment at two different dosages were significantly ameliorate ACh-induced aortic relaxation without modulating MAP of male L-NAME hypertensive Wistar rats.

Conflict of Interest

There is no conflict of interest concerning the publication of the article.

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