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A quinazoline-derivative compound with PARP inhibitory effect suppresses hypertension-induced vascular alterations in spontaneously hypertensive rats

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ABSTRACT

Aims: Oxidative stress and neurohumoral factors play important role in the development of hypertension-induced vascular remodeling, likely by disregulating kinase cascades and transcription factors. Oxidative stress activates poly(ADP-ribose)-polymerase (PARP-1), which promotes inflammation and cell death. We assumed that inhibition of PARP-1 reduces the hypertension-induced adverse vascular changes. This hypothesis was tested in spontaneously hypertensive rats (SHR).

Methods and results: Ten-week-old male SHRs and wild-type rats received or not 5 mg/kg/day L-2286 (a water-soluble PARP-inhibitor) for 32 weeks, then morphological and functional parameters were determined in their aortas. L-2286 did not affect the blood pressure in any of the animal groups measured with tail-cuff method. Arterial stiffness index increased in untreated SHRs compared to untreated Wistar rats, which was attenuated by L-2286 treatment. Electron and light microscopy of aortas showed prominent collagen deposition, elevation of oxidative stress markers and increased PARP activity in SHR, which were attenuated by PARP-inhibition. L-2286 treatment decreased also the hypertension-activated mitochondrial cell death pathway, characterized by the nuclear translocation of AIF. Hypertension activated all three branches of MAP-kinases. L-2286 attenuated these changes by inducing the expression of MAPK phosphatase-1 and by activating the cytoprotective PI-3-kinase/Akt pathway. Hypertension activated nuclear factor-kappaB, which was prevented by PARP-inhibition via activating its nuclear export.

Conclusion: PARP-inhibition has significant vasoprotective effects against hypertension-induced vascular remodeling. Therefore, PARP-1 can be a novel therapeutic drug target for preventing hypertension-induced vascular remodeling in a group of patients, in whom lowering the blood pressure to optimal range is harmful or causes intolerable side effects.

Abbreviations: AngII, angiotensin II; AIF, apoptosis inducing factor; ASI, Aortic stiffness index; AP-1, activator protein 1; ASK1, apoptosis signal-regulating kinase 1; Crm1, chromosome region maintenance 1; DAB, 3,3′-diaminobenzidine; DBP, diastolic blood pressure; DD, diastolic diameter; ERK, extracellular signal regulated kinase; GSK-3β, glycogen synthase kinase 3; 4-HNE, 4-hydoxy-2-nonenal; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; p38 MAPK, p38 mitogen-activated protein kinase; MKP-1, MAP kinase phosphatase-1; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappaB; PAR, poly-ADP ribosylation; PARP-1, poly(ADP-ribose)polymerase-1; PBS, phosphate buffered saline; PI-3 kinase, phosphatidylinositide 3-kinase; PKC, protein-kinase C; ROS, reactive oxygen species; SBP, systolic blood pressure; SD, systolic diameter; SEM, standard error of the mean; SHR, spontaneously hypertensive rat; STAT, Signal transducer and activator of transcription; TGF-β1, transforming growth factor beta 1; VEGF, vascular endothelial growth factor; WKY, Wistar Kyoto rats

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1. Introduction

Hypertension is a major public health problem both in middle-aged and elderly people. It is both a complex disease and an important risk factor for other cardiovascular outcomes, such as sudden death, stroke, myocardial infarction, heart failure, and renal diseases [1]. Unfortunately, the control of arterial hypertension is far from optimal and has improved only minimally over the last decades [1]. Side effects of antihypertensive drugs, complaints due to their blood pressure lowering effect and inadequate compliance are the key factors in the background of inadequate control of hypertension [1,2]. Moreover lowering blood pressure to the optimal range can be harmful in elderly patients [3,4].

The spontaneously hypertensive rat (SHR) model is a suitable model for studying the development and consequences of hypertension. The development of vascular remodeling is an early and important consequence of hypertension. Vascular remodeling is mainly characterized by vascular smooth muscle cell hypertrophy and increased production of extracellular matrix [5]. Remodeling is initially an adaptive process that evolves in response to long-term pressure overload, but finally it can contribute to the development of hypertensive target organ damages [3–5].

By now it has been well established that oxidative stress has a causative role in the development of hypertensive vascular complications and vascular remodeling [5,6]. ROS are important in regulating endothelial function and vascular tone, and are implicated in endothelial dysfunction, inflammation, hypertrophy, apoptosis, migration, fibrosis and angiogenesis [7,8]. ROS-induced injury of the ion channels and the decreased amount of high energy phosphates can result in profound alterations in smooth muscle cell calcium homeostasis leading to increased smooth muscle cell proliferation and contractility [9]. The increased vascular tensile stress, the oxidative stress and neurohumoral factors in hypertension equally influence the activity of downstream signaling molecules [10,11]. Oxidative stress induced signaling such as activation of MAPKs, PKC isoenzymes, NF-κB, AP-1 and STAT can significantly contribute to the development of hypertension induced vascular remodeling [10,12–14]. Therefore, attenuating oxidative stress, or inhibiting these pathways may present an alternative way to protect the vasculature from hypertension induced damages [5,14]. Using antioxidants did not produce the expected results [5,15] necessitating specific inhibition of ROS-induced processes that lead to signaling anomalies.

Oxidative stress induced by hypertension can lead to single stranded DNA breaks and poly(ADP-ribose)polymerase-1 (PARP-1) activation [16,17]. Because of its high copy number, overactivation of the enzyme can induce NAD^+ and thereby ATP depletion leading to necrotic cell death [18–20], or trigger apoptosis by promoting the release of mitochondrial pro-apoptotic proteins, such as AIF and endonuclease G [22,23]. In addition, PARP activation by promoting mitochondrial damage [21] can further induce ROS production, elevated intracellular Ca^{2+} and destabilize the mitochondrial membrane system leading to mitochondrial permeability transition and cell death [20,22,34]. Furthermore, PARP activation can activate NF-κB and AP-1 transcription factors [24–26], which can significantly contribute to cardiovascular remodeling [27,28]. Therefore, it is likely that inhibition of PARP can favorably influence intracellular signaling during hypertension, which can contribute to the reduction of vascular remodeling [29]. Activation of PARP was demonstrated within vascular endothelial and smooth muscle cells of large atherosclerotic arteries [30,31]. Inhibition of PARP, in turn, had a positive effect on vascular dysfunction associated with advanced aging, diabetes or hypercholesterolemia [7,8,30,31]. Furthermore, as we found previously, inhibition of PARP by L-2286 [32] influenced beneficially the signaling pathways in hypertension induced cardiac remodeling [28].

However, there is no available data concerning the potential role of PARP activation in the pathogenesis of hypertension-induced vascular dysfunction and remodeling in vivo. On the basis of the aforementioned, we hypothesized that the administration of L-2286 in hypertensive animals decreases oxidative stress, modulates signaling pathways (PI-3K-Akt, MAP kinases) and attenuates NF-κB activation, thereby reduces the hypertension-induced adverse vascular changes. In the present study we tested this hypothesis by using spontaneously hypertensive rats.

2. Methods

2.1. Animal model and noninvasive blood pressure measurement

Ten-week-old male SHR rats obtained from Charles River Laboratories (Budapest, Hungary) were randomly divided into two groups. One group received no treatment (SHR-C, n = 10), whereas the other group (SHR-L, n = 10) received 5 mg/kg/day 2-[(2-piperidine-1-ylethyl)thio]quinazolin-4(3H)-one (L-2286), a water-soluble PARP inhibitor [32] for 32 weeks. The third group was an age-matched normotensive control group (WKY-C, n = 10, Charles River Laboratories, Budapest, Hungary). The fourth was a normotensive age-matched group receiving 5 mg/kg/day L-2286 treatment (WKY-L, n = 10). The dose of L-2286 was based on our previous results with this PARP inhibitor [28,31]. According to these data, L-2286 can exert protective effects against oxidative cell damage in concentration of 10 μM. The serum concentration of L-2286 in the applied dose (5 mg/kg/day) with an estimated average bioavailability is approximately 10 μM in rats. L-2286 was dissolved in drinking water on the basis of preliminary data about the volume of daily consumption, but the water was provided ad libitum throughout the experiment. Before the administration of L-2286 and at the end of the 32-week treatment period, ultrasound imaging was performed on each animal. Noninvasive blood pressure measurement was carried out in every fourth week from the beginning of the study using the tail-cuff method (Hatteras SC 1000 Single Channel System) [33]. At the end of the study, animals were euthanized with an overdose of ketamine hydrochloride intraperitoneally and heparinized with sodium heparin (100 IU/rat i.p., Biochemie GmbH, Kundl, Austria). Aortas were removed under an Olympus operation microscope and were freeze-clamped and stored at −70 °C or fixed in 10% formalin. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and was approved by the Animal Research Review Committee of the University of Pecs Medical School.

2.2. Transthoracic echocardiography

At baseline, all animals were examined by ultrasound to exclude rats with any abnormalities. Two-dimensional ultrasound was performed under inhalation anesthesia at the beginning of the experiment and on the day of sacrifice. Rats were lightly anesthetized with a mixture of 1.5% isoflurane and 98.5% oxygen. The necks and the upper part of the chests of the animals were shaved, acoustic coupling gel was applied, and warming pad was used to maintain normothermia. ASI were measured by a VEVO 770 high-resolution ultrasound imaging system (VisualSonics, Toronto, Canada) — equipped with a 40 MHz transducer. Aortic elastic property was calculated according to a previously proposed and evaluated formula [34]: $(ASI) = \ln(SBP/DBP) \times DD/(SD - DD)$.

2.3. Immunohistochemistry and confocal laser scanning fluorescence microscopy

Aortic segments separated for immunohistochemical and immunfluorescence processing were fixed immediately after excision in buffered paraformaldehyde solution (4%) for 1 day. Five mircometer thick sections were cut.