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DISSERTATION

New approaches to the treatment of target organ damage in experimental models of hypertension.

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"The treatment of hypertension itself is a difficult and almost hopeless task in the present state of knowledge, and in fact for aught we know...the hypertension may be an important compensation mechanism which should not be tampered with, even were it certain that we could control it."

—Paul Dudley White, 1937

Abstract

Hypertension and the associated target organ damage remain uncontrolled in many affected patients despite receiving an optimal therapy. This has spurred the search for new agents that can effectively control blood pressure and reduce target organ damage.

The aims of this work were to examine protective effects of three new pharmacological agents representing different pathways for antihypertensive treatments. We analyzed the effects of riociguat, a novel stimulator of the soluble guanylate cyclase (sGC); SLV338, the combined inhibitor of neutral endopeptidase (NEP) and endothelin-converting enzyme (ECE); and the novel adenosine A_1 receptor antagonist, SLV320, in experimental models of hypertension and chronic renal failure.

The cardio- and renoprotective effects of riociguat were evaluated in hypertensive renin-transgenic rats with additional blockade of the nitric oxide (NO) system by the nitric oxide-synthase inhibitor, N-nitro-L-arginine methyl ester (L-NAME) (high-renin model), and in rats with 5/6 nephrectomy (NX) (low-renin model). The cardioprotective actions of SLV338 were evaluated in experimental renovascular hypertension (2-kidney, 1-clip [2K1C] model).The protective effects of SLV320 were examined in the heart and kidney of rats with 5/6 nephrectomy (NX).

Riociguat treatment improved the survival rate, normalized blood pressure, improved renal function and significantly ameliorated fibrotic tissue remodeling in the heart and kidney in the low- and high-renin models.

Treatment with the dual ECE/NEP inhibitor SLV338 completely normalized cardiac interstitial fibrosis, perivascular fibrosis, myocyte diameter and media-to-lumen ratio of cardiac arteries, and it attenuated cardiac transforming growth factor- ß1 expression. These effects were similar to those of losartan. The observed cardioprotective benefits of SLV338 were independent of blood pressure.

The A_1 receptor antagonist SLV320 significantly decreased cardiac fibrosis and albuminuria in rats with 5/6 NX. These protective effects occurred without changes in blood pressure.

In summary, these results demonstrate that the sGC stimulator riociguat, the combined inhibitor of NEP/ECE SLV338, and the adenosin A_1 receptor antagonist SLV 320 play important roles in cardio-renal protection in experimental models of hypertension and chronic renal failure. In the future, these approaches need to be balanced with the established beneficial effects of current antihypertensive therapies.

Abstract (deutsch)

Arterielle Hypertonie und die damit assoziierten Organschäden bleiben bei vielen betroffenen Patienten unkontrolliert, obwohl sie eine optimale Therapie erhalten. Die Entwicklung neuer, wirksamerer Arzneimittel zur Kontrolle des Blutdrucks ist daher nach wie vor erforderlich. Das Ziel dieser Arbeit war die Auswertung der protektiven Effekte dreier, sich momentan in der Entwicklungsphase befindender Pharmaka. Es wurden Substanzen ausgewählt, die in verschiedene Signalketten eingreifen: Riociguat, ein neuer Stimulator der löslichen Guanylatcyclase (sGC); SLV338, ein kombinierter Hemmer der neutralen Endopeptidase (NEP) und des Endothelin converting enzyme (ECE); SLV 320, ein Adenosin-A_1-Rezeptorantagonist. Die protektive Wirkung dieser Substanzen auf hypertensive Organschäden und chronische Niereninsuffizienz wurde mit Hilfe von tierexperimentellen Modellen der Hypertonie evaluiert.

Kardio- und nephroprotektive Wirkungen von Riociguat wurden an hypertensiven Ratten mit Überexpression von Renin und zusätzlicher Blockade des Stickstoffmonoxid (NO) Systems durch N-nitro-L-arginine methyl ester (L-NAME), einem hoch-Renin-Modell, und an Ratten mit 5/6 Nephrectomie (NX), einem niedrig-Renin-Modell untersucht. Organprotektive Effekte von SLV320 auf Herz und Nieren wurden an Ratten mit 5/6 NX evaluiert. Die kardioprotektive Effekte von SLV338 wurden am Modell der renovaskulären Hypertonie (2-kidney, 1-clip [2K1C]) untersucht.

Die Therapie mit Riociguat führte zur Verbesserung der Überlebensrate, Normalisierung des Blutdrucks, Verbesserung der Nierenfunktion und zu einer signifikanten Verringerung der Fibrose in Herz und Nieren sowohl im niedrig- als auch im hoch-Renin-Modell.

Die Therapie mit SLV338 führte zur vollständigen Normalisierung der interstitiellen Fibrose des Herzens, der perivaskulären Fibrose, des Myozytendurchmessers, des Media-Lumen-Verhältnisses von Herzkranzgefäßen, sowie zur Senkung der Expression von Transforming growth factor- ß1 bei hypertensiven 2K1C-Ratten. Ähnliche Effekte wies auch Losartan auf. Die kardioprotektiven Effekte von SLV338 waren blutdruckunabhängig.

Die Therapie mit SLV320 führte zur signifikanten Reduktion der Herzfibrose und der Albuminurie bei Ratten mit 5/6 NX; diese Effekte waren nicht mit Veränderungen des Blutdrucks assoziiert.

Zusammenfassend zeigen diese Resultate, dass der Stimulator der sGC, Riociguat, der kombinierte Hemmer der NEP und des ECE, SLV338, und der Antagonist des A_1 Rezeptors, SLV320, wichtige Rollen in der Kardio- und Nephroprotektion bei experimenteller arterieller Hypertonie spielen können. Allerdings müssen die drei untersuchten Pharmaka zukünftig mit den positiven Effekten der aktuell etablierten antihypertensiven Therapien abgeglichen werden.

Abbreviations

ALT	alanine amino transferase
ANG I	angiotensin I
ANG II	angiotensin II
ANP	atrial natriuretic peptide
AST	aspartate amino transferase
AT_1	angiotensin II type 1 receptor
BNP	brain natriuretic peptide
cGMP	cyclic guanosine monophosphate
CK	creatine kinase
CNP	C-type natriuretic peptide
CrCl	creatinine clearance
ECE	endothelin converting enzyme
ET-1	endothelin-1
ETA	endothelin receptor A
ETB	endothelin receptor B
GFR	glomerular filtration rat
GLDH	glutamate dehydrogenase
GTN	glyceryl trinitrate
HE	hematoxylin-eosin
2K1C	two-kidney, one-clip
LDH	lactate dehydrogenase
L-NAME	NG-Nitro-L-arginine methyl ester
NEP	neutral endopeptidase
NX	nephrectomy
NO	nitric oxide
PRA	plasma renin activity
RAS	renin–angiotensin system
SBP	systolic blood pressure
sGC	soluble guanylate cyclase
TGF-ß1	transforming growth factor- ß1

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1. Introduction

Many cardiovascular and renal diseases are regarded as a continuum that starts with risk factors, such as hypertension and dyslipidemia and progresses to atherosclerosis and clinical syndromes such as myocardial infarction. These events produce persistent end-organ damage and ultimately lead to heart failure, end-stage renal disease, stroke or death, representing the most common cause of morbidity and mortality in industrial countries (1). Besides atherothrombotic diseases like stroke or myocardial infarction, the development of heart failure is a major contributor to cardiovascular mortality. Moreover, very recently the ACCORD trial demonstrated that even in a cardiovascular high risk population of diabetic patients there is no effect of further blood pressure lowering on cardiovascular outcomes (2). Thus, novel therapeutic strategies beyond blood pressure lowering to halt cardiac remodelling are vital.

The vascular endothelium synthesizes and releases a spectrum of vasoactive substances that modulate vascular tone, homeostasis, inflammatory responses and angiogenesis. The vasoactive factors include relaxing (nitric oxide, prostacyclin, adenosine, C-natriuretic peptide, etc.) and contracting factors (thromboxane A2, endothelin-1, angiotensin II, superoxide anion, etc.). In a number of cardiovascular pathologies, such as hypertension or heart failure, the balance in the endothelial production of vasodilating and vasoconstricting mediators is altered.

In the first part of the work we assessed the cardio-renal protective effects of riociguat in two rat models of systemic hypertension. Nitric oxide (NO) is considered an important vasodilator. Nitric oxide achieves its effects mainly by acting on soluble guanylate cyclase (sGC), leading to increased production of the second messenger cyclic guanosine monophosphate (cGMP). Therefore, pharmacological stimulation of sGC is an appealing strategy to treat hypertensive cardiac and renal target organ damage. Unlike nitric oxide-donor drugs, stimulators of sGC directly stimulate the native form of the enzyme to increase cGMP production and make sGC more sensitive to the effects of the body's own nitric oxide (3, 4). However, little is known about the effects of sGC stimulators in experimental models of hypertension. We thus investigated the cardio-renal protective effects of riociguat (BAY 63-2521) in low-renin and high-renin rat models of hypertension. The 5/6 nephrectomy (NX) model was used as a low-renin model for chronic renal failure with moderate hypertension. For the high-renin model, we used the renin-transgenic rats [TG(mRen2)27] rats which express an additional mouse rennin gene and develop malignant hypertension in conjunction with an activated adrenal renin-angiotensin system.

In the second part of the work we investigated the beneficial effects of SLV338, a novel endothelin-converting enzyme (ECE)/neutral endopeptidase (NEP) inhibitor in a rat model of renovascular

hypertension. Endothelin-1 (ET-1) is the foremost vasoconstrictor factors (5) that exhibits profibrotic and proinflammatory properties (6). It is derived from a biologically inactive precursor, Big-ET-1, by the action of ECE (7). Since clinical trials with endothelin receptor antagonists in heart failure produced disappointing results, which were probably due to complex systemic actions of these antagonists, pharmacological blockade of ET-1 synthesis, by inhibition of ECE, is an intuitive strategy in reducing the elevated activity of ET-1 associated with numerous cardiovascular disease states (8). Another approach to curbing hypertensive cardiac remodeling is to enhance plasma levels of vasodilatory, antihypertrophic and antifibrotic mediators like the natriuretic peptides (atrial natriuretic peptide, brain natriuretic peptide and C-type natriuretic peptide) (9) by inhibiting their degradation via the NEP. Moreover, it is known that NEP degrades ET-1. The conversion of big-ET-1 to ET-1 occurs via a unique processing event catalyzed by ECE, which has been reported to be a zinc-dependent metalloendopeptidase homologous with NEP (10; 11). Therefore, the rationale for creating combined ECE/NEP inhibitors is twofold: combined ECE/NEP inhibition reduces ET-1 production and unveils the beneficial effects of NEP inhibition via increased natriuretic peptide signaling without a concomitant increase (or even with a decrease) of ET-1 levels. Moreover, blockade of the ET-system leads to salt and fluid retention due to the role of Endothelin B receptors (ETB) in sodium handling in the kidney (12; 13), which has been a major drawback in clinical studies. Combination with NEP inhibition and subsequent increase of natriuretic peptide action might be a feasible way to overcome this problem. However, the potential of combined ECE/NEP inhibition in myocardial protection has not been fully explored so far. In the second part of the work we examined in vitro and in vivo features of SLV338, a novel ECE/NEP inhibitor, with special focus on the prevention of cardiac remodeling in a model of renovascular hypertension in comparison to standard end organ protection with losartan.

In the third part of our work we examined the protective effects of the selective adenosine A1 receptor antagonist SLV320 on target organ damage in rats with 5/6 NX. The adenosine system is involved in several key functions of both kidney and heart. Adenosine acts via four different receptors: A1, A2A, A2B and A3 (14). In the heart, A1 receptor activation may be harmful in ischaemia/reperfusion injury, for instance, through promotion of neutrophil chemoattraction and adhesion (15, 16, 17), whereas A2 and/or A3 activation is protective in this setting. In the kidney, adenosine plays a key role in the tubuloglomerular feedback mechanism, and thus exerts an inhibitory action on glomerular haemodynamics and glomerular filtration rate (GFR) via A1 receptors (18, 19). Moreover, adenosine has anti-natriuretic (and anti-diuretic) effects, through activation of tubular A1 receptors, which promote sodium resorption (20, 21, 22). In patients with congestive heart failure, A1 receptor antagonists exhibit the intriguing features of increased diuresis

and GFR, whereas the loop diuretic furosemide increases diuresis at the expense of a decreased GFR (23). In addition, animal studies show that A1 receptor antagonists display renoprotective properties in the setting of radiographic contrast medium-induced nephropathy (24), as well as in cisplatin-induced renal failure (25). Given these scientific data, further studies addressing the cardiac effects of A1 adenosine antagonists are urgently needed. In the third part of the work we analyzed the novel selective adenosine A1 receptor antagonist SLV320 focusing on its potential in preventing cardiomyopathy in a rat model of chronic renal failure.

1.1. Aims of the study

We hypothesized that novel pharmacogenetic approaches to hypertension therapy such as pharmacological stimulation of sGC, combined inhibition of NEP with ECE and blockade of an adenosine A1 receptor may prevent hypertensive end-organ damage in experimental models of hypertension and chronic renal failure.

The aims were therefore to examine the

- cardio-renal effects of pharmacological stimulator of sGC (riociguat) in independent rat models of low-renin and high-renin hypertension.
- protective effects of combined NEP/ECE on cardiac remodelling in a rat model of renovascular hypertension.
- protective effects of the blockade of an adenosine A1 receptor in the heart and kidney in rats with 5/6 NX.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all other reagents were of analytical grade and were purchased from Sigma (Seelze, Germany), Merck (Darmstadt, Germany) and Roth (Karlsruhe, Germany).

Riociguat [BAY 63-2521; methyl 4,6-diamino-2-[1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl]pyrimidin-5-ylmethylcarbamate] was synthesized (developed) by Bayer Schering Pharma (26, 27).

Riociguat constitutes the first drug of a novel class of sGC stimulators.

SLV320 and SLV338 were synthesized by Solvay Pharmaceuticals, now Abbott Products GmbH (Hanover**,** Germany).

SLV338 is a novel ECE/NEP inhibitor.

SLV320 is a selective A1 Adenosine Receptor Antagonist.

2.2. Animal models

All animal studies were carried out in accordance with the German and French legislation on the use of laboratory animals.

2.2.1. The in-vitro effect of riociguat in isolated rabbit saphenous artery

The vasorelaxant effect of riociguat and glyceryl trinitrate has been examined on isolated saphenous artery rings taken from normal and nitrate-tolerant chinchilla rabbits as reported previously (27).

New Zeeland white rabbits of either sex (weight = 2-3 kg) were used. The skin was shaved between both scapulae. To induce nitrate tolerance, isosorbidedinitrate (ISDN, TD Spray Iso Mack®, Heinrich Mack, Illertissen, Germany) was applied percutaneously with a dose of approximately 150-250 mg 3 three times a day over a period of 4 days (28). These rabbits were then sacrificed by an overdose of thiopental. Saphenous artery rings (3 mm width) were suspended under an initial tension of approximately 4g in 5ml organ baths containing Krebs–Henseleit solution at 37°C. Contractions were measured isometrically with Statham UC2 strain gauges connected to a DAS1802HC data acquisition board (Keithley Instruments, Germering, Germany). Rings were precontracted by 3×10^{-8} g/ml phenylephrine (submaximal contraction) four times. Each contraction was followed by a series of 11 washing cycles and a resting period of 30 min. Then, a tonic contraction was induced by phenylephrine 3×10^{-8} g/ml. The contraction was allowed to stabilize, before the test compounds were added to the organ bath in a cumulative manner. The concentration of the test compounds was increased by a factor of 10.

2.2.2. Receptor binding and enzyme assays of SLV338 and SLV320

The receptor binding affinities and enzyme inhibitory properties of SLV338 and SLV320 were evaluated in a broad panel of receptors and ion channels by use of standard protocols (Cerep, Celle l'Evescault, France).

For receptor binding assays, the inhibition constants were calculated using the Cheng-Prushoff equation. Results were expressed as mean p inhibition constant values ± SD of ≤ 2 separate experiments done in duplicate.

2.2.3. In vitro and in vivo assessment of ECE and NEP inhibition by SLV338

The potency of SLV338 to inhibit the enzymatic activities of NEP (E.C. 3.4.24.11) and ECE-1 (E.C. 3.4.24.71) was investigated in an assay using recombinant human enzymes and modified 17 amino acid truncated Big-ET-1 as substrate. This peptide substrate contains a fluorescence label (Mca =

7-methoxycoumarin-4-yl) at its N-terminus, and the quencher (Dpa = 3-[2,4-dinitrophenyl]-L-2,3-diaminopropionyl) in its internal sequence. Because NEP and ECE-1 cleave this peptide, their activity leads to an unquenching and, thus, quantifiable increase of the 7-methoxycoumarin-4-yl fluorescence.

To evaluate the efficacy and potency of SLV338 with regard to the compound's ability to inhibit ECE in vivo, the effect of SLV338 to prevent the acute blood pressure response to Big-ET was examined in anesthetized male Sprague Dawley rats, based on the notion that ECE inhibition reduces the conversion of Big-ET-1 to the active vasoconstrictor ET-1. After i.p. anaesthesia with ketamine/xylazine, a pressure transducer (Statham) was connected to one carotid artery for measurement of arterial blood pressure. Both jugular veins were cannulated for drug- and Big-ET-infusion, respectively. After an equilibration period of 20 min, the animals received SLV338, given at the indicated doses (expressed as µmol/kg) or vehicle i.v. (over 1 min in a total volume of 2 mL/kg body weight). Five minutes later 0.5 nmol/kg of human Big-ET was infused (over 1 min; Big-ET was purchased from Bachem, Heidelberg, Germany). Blood pressure and heart rate were recorded every 5 min for the next 15 min. The increase in blood pressure was expressed as percentage of response to Big-ET-1 in vehicle-treated animals.

NEP inhibition by SLV338 in vivo was assessed by taking advantage of the fact that the known degradation route for ANP is via NEP. Accordingly, NEP inhibition increases ANP plasma levels and enhances ANP-dependent natriuresis and urinary cGMP-excretion. Functional inhibition of NEP by SLV338 in vivo was examined using anesthetized male Sprague Dawley rats. Catheters were placed into one jugular vein for vehicle or SLV338 administration, into the contralateral jugular vein for ANP infusion, and into the bladder for urine collection and assessment of natriuresis and urinary cGMP levels.

After equilibration following initiation of anesthesia 2 mL 0.9% NaCl was slowly administered i.v., followed by a continuous infusion of 62.5 µL 0.9% NaCl/min for 60 minutes. 30 min after the start of this volume load, rats were treated with SLV338 by bolus injection and subsequent continuous infusion as follows: A slowly applied intravenous bolus injection of SLV338 of 1 mg/kg in 1 mL vehicle per kg, followed by continuous intravenous infusion of SLV338 at a constant rate of 33 µg/kg.min in 20 µL per minute until the end of the experiment. Fifteen min later, a continuous intravenous administration of rat ANP started at a rate of 300 ng/kg.min in 62.5 µL 0.9% NaCl per minute for a total duration of 60 minutes, followed by continuous infusion of 62.5 µL 0.9% NaCl per minute until the end of the experiment. Overall, rats thus received a mean total volume load of approximately 5 mL per hour. Blood samples were taken for measurement of ANP levels (at the end of the ANP infusion).

2.2.4. Haemodynamic effects of SLV320

Male CD rats (220–290 g body weight, Charles River, Wiga, Wilmington, MA, USA) were used for the experiments. Under Trapanal anesthesia, the trachea and one carotid artery and jugular vein were cannulated to monitor blood pressure and heart rate and to infuse adenosine, respectively. The arterial catheter was connected to a pressure transducer. Needle electrodes were fixed to the right forelimb and left hindlimb and the sternal region for ECG recording. Heart rate and blood pressure were measured every 10 s throughout the study (and every 2 s for 1 min following each adenosine challenge); heart rate values were averaged over 1 min preceding and over 1 min following each adenosine challenge. The heart rate reduction by adenosine after each SLV320 dose was expressed as percentage of the mean predrug rate reduction (that is the average value of the response measured in the first two adenosine challenges).

2.2.5. The experimental study of riociguat's cardio-renal protective effects in low-renin and high-renin rat models of hypertension (riociguat)

2.2.5.1. High-renin study with renin-transgenic NG-nitro-L-arginine methyl ester (L-NAME) -treated rats (malignant hypertension)

We used forty-eight week old male renin-transgenic rats [TG(mRen2)27]. These animals represent a malignant model of hypertension with a high hypertension-induced mortality within the first few weeks after starting application of the nitric oxide-synthase inhibitor, N-nitro-L-arginine methyl ester (L-NAME). L-NAME was chronically administered via drinking water (100 mg/l) in all study groups. Animals were randomly allocated to three study groups: control (n=24), riociguat low dose, and riociguat high dose (3 and 10 mg/kg per day, respectively, administered per os by gavage once daily, n=12 per group). Blood pressure was measured via tail-cuff method once before the start of the study (day 0) to exclude preexisting differences between the groups and on day 4 and 16. Body weight and survival was assessed on day 1, 8, 15 and at study end. At the end of the study (day 18), all animals were sacrificed, blood was taken in order to assess plasma parameters, and hearts and kidneys were weighed and harvested for histological evaluation.

2.2.6. Low-renin study with 5/6 NX Wistar rats (moderate hypertension)

Rats with 5/6 NX were used as low-renin model of hypertension. This low-renin study lasted 18 weeks and contained 3 groups: 5/6 NX (n=15), 5/6 NX plus treatment with riociguat (300 ppm in the solid feed, resulting in approximately 15 mg/kg per day, n=15) and sham-operation (n=10). During the study period, the animals were weighed weekly and blood pressure was assessed via

the tail-cuff method during weeks 0, 2, 5, 9 and 15. The animals were placed into metabolic cages to obtain 24 h urine samples at weeks 0, 4 and 17. At the same time, blood was taken from retro-orbital veins for the purpose of measuring plasma creatinine levels and calculating creatinine-clearance using standard formula (11). At the end of the study (week 18), animals were sacrificed in deep narcosis by permanent inhaled isofluorane via aortic puncture, animals were bled and blood samples were taken for plasma analysis. The hearts and kidneys were harvested for histological studies and the organs' weights were measured.

2.2.7. In vivo assessment of cardioprotective properties of SLV338 in renovascular hypertensive rats ('Two-kidney, one-clip' (2K1C))

Rats were subjected to unilateral clipping of the renal artery in order to produce renovascular hypertension (29) in a 2K1C model. In brief, after a 2 week acclimatization period, male Sprague Dawley rats (205 - 283g) were anesthetized using ketamine/xylazine 100/10 mg/kg i.m. A U-shaped silver clip (0.20 mm internal diameter) was placed around the left renal artery through a dorsal flank incision. The right kidney was not disturbed. Sham animals underwent the same procedure without clipping. Afterwards animals were allocated to five groups: sham surgery (Sham; n=6), untreated 2K1C (2K1C; n=12), 2K1C plus SLV338 30 mg/kg/d (2K1C+SLV338 low dose; n=12), 2K1C plus SLV338 100 mg/kg/d (2K1C+SLV338 high dose; n=12) and 2K1C plus Losartan 20 mg/kg/d (2K1C+ LO; n=12). All animals received a standard powdered rat diet with tap water ad libitum during the study period. SLV338 was administered orally (mixed with food at 30 and 100 mg/kg.d) for 12 weeks, starting 2 weeks (week 0) after left kidney clipping. The angiotensin AT_1 receptor antagonist losartan (20 mg/kg/d), was administered under the same conditions. Systolic blood pressure (SBP, mmHg) was measured via tail cuff method (BP-2000, Visitech Systems, Bioseb, France) 2 weeks after surgery (week 0) and during weeks 4, 8 and 12 after the start of the treatment. Only 2K1C rats with SBP greater than 150 mmHg at week 0 were included in the study protocol. At the end of the experiment (i.e. in Week 12) the animals were sacrificed under isoflurane anesthesia, and hearts were harvested for further studies.

2.2.8. The experimental study of SLV320 protective effects in a rat model of chronic renal failure 5/6 NX and uraemic cardiomyopathy

After a 2-week recovery period, the left kidney was exposed and 2/3 was surgically removed. The sham operated animals underwent the same operations, but kidneys were only mobilized instead of being removed. Male Sprague-Dawley rats were allocated to 4 groups of 12 rats each: 5/6 NX, 5/6 NX plus SLV320 ($10 mgkg^{-1}$ d^{-1} mixed with food), sham and sham plus SLV320. Study duration

was 12 weeks, blood pressure was assessed repeatedly. At study end kidney function was assessed, blood samples and hearts were taken for histology/immunohistochemistry.

2.3. Laboratory chemistry

Aspartate amino transferase (AST), alanine amino transferase(ALT), glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), creatine kinase (CK), creatinine, urea and total protein were measured in plasma as described before (30). Plasma renin activity (PRA), atrial natriuretic peptide (ANP) and angiotensin I (ANG I) in plasma were determined after extraction as previously described (27, 31).

2.3.1. Riociguat plasma levels

Plasma concentrations of riociguat were measured by high-performance liquid chromatography (2300HTLC; Cohesive Technologies, Franklin, Massachusetts, USA) and tandem mass spectrometry (API 3000; PE Sciex, Concord, Ontario, Canada) as previously described (32).

2.4. Histological studies

Tissue samples were all embedded in paraffin, cut into 3 µm sections, subjected to Sirius Red, Elastica–van Gieson and hematoxylin-eosin (HE) staining. Quantitative histomorphometry (i.e. intima/media and lumen area of the arteries, interstitial fibrosis and myocyte diameter) was analyzed using a computer-aided image analysis system as previously described (12; 30).

2.5. Immunohistochemistry

2.5.1. SLV338

Paraffin tissue sections were incubated with Rabbit anti-TGF-ß1 antibody (Acris Antibodies GmbH, Germany). Afterwards the sections were incubated with biotinylated goat anti-rabbit secondary antibody (DAKO, Denmark) and then treated with Vectastain Elite ABC Kit (Avidin/Biotin/Horseradish Peroxidase-System; Vector Laboratories, USA). Visualization of peroxidase reaction was achieved using 3-amino-9-ethylcarbazole (AEC, DAKO) chromogen solution. Finally, the slides were counterstained with Hematoxylin. Negative controls were prepared by leaving out the primary antibodies. Transformin growth factor (TGF-ß1) staining was quantified by image analysis as described for interstitial fibrosis measurement above. Results are expressed as percent area positive for TGF-ß1.

2.5.2. SLV320

Frozen tissue sections were incubated simultaneously with polyclonal rabbit antibodies against collagen types I and III as well as fibronectin and laminin. Detection of the bound antibodies was performed using a biotinylated second antibody and streptavidin Texas red according to the manufacturer's instructions (Amersham Buchler, Braunschweig, Germany). Control experiments were performed omitting the first antibody and using phosphate-buffered saline instead. Matrix protein expression (matrix protein-positive area in relationship to total area of interest) was quantitatively analysed using a computer-aided image analysis system (see above).

2.6. Statistical analysis

Differences between the groups were compared using ANOVA or Kruskal-Wallis tests (for normally or non-normally distributed data) as appropriate. The Student's t- or Mann–Whitney U tests were used to detect significant differences between 2 groups of interest. Results were expressed as mean ± standard error of the mean; differences were considered significant when the probability error (p) was less than 0.05. Survival analysis was carried out using Kaplan–Meier analysis and the log–rank test.

3. Results

3.1. Riociguat study

The vasorelaxant effect of riociguat has been examined on isolated saphenous artery rings taken from normal and nitrate tolerant rabbits. The cardio- and renoprotective effects of riociguat were evaluated in two rat models of systemic hypertension: a high-renin model (L-NAME treated renin-transgenic rats) and a low-renin model (rats with 5/6 NX).

3.1.1. Effect of riociguat on isolated precontracted saphenous arteries

The relaxant effect of riociguat was examined on isolated artery rings taken from normal and nitrate-tolerant chinchilla rabbits. Glyceryl trinitrate (GTN) inhibited the phenylephrine-induced contraction with IC_{50} = 13.2 nmol/l (95% confidence limits from 7.8 to 22.1 nmol/l) in control vessels and with IC_{50} = 65.1 nmol/l (95% confidence interval from 46.8 to 92.8 nmol/l) in tolerant vessels, confirming the presence of nitrate tolerance. In contrast, riociguat relaxed saphenous arteries which were taken from normal and tolerant rabbits with an IC_{50} = 4.8 nmol/l (with 95% confidence limits from 2.1 to 8.5 nmol/l) and 5.6 nmol/l (with 95% confidence limits from 1.6 to 12.7 nmol/l), respectively.

3.1.2. The experimental study of riociguat's cardio-renal protective effects in low-renin and high-renin rat models of hypertension (riociguat)

3.1.2.1. Survival

In the high-renin study part, the beneficial effects of riociguat are emphasized by a significantly increased survival rate in both dosages (riociguat low dose: 92%, riociguat high dose: 100% vs 46% in L-NAME-treated renin transgenic rats). In the low-renin study part, overall survival was higher: 5/6 NX 60%, 5/6 NX + riociguat 73%, sham 100%.

3.1.2.2. Body and organ weight

Body weight at study end did not significantly differ between the L-NAME treated groups (Kruskal-Wallis, P = 0.25). Left cardiac ventricle weight was significantly decreased in rats treated with high dose riociguat in comparison to control and low dose riociguat-treated animals, whereas the relative weight of the entire heart remained unchanged.

Both nephrectomized groups weighed significantly less than sham-operated group. Left cardiac ventricle weight increased significantly in both nephrectomized groups compared to sham-operated controls. However, this effect was markedly attenuated by treatment with riociguat.

3.1.2.3. Blood pressure

SBP was markedly increased in control animals under L-NAME treatment and in both nephrectomized groups compared with sham-operated controls. In both models the blood pressure was significantly reduced by riociguat. The increase in SBP in 8-week-old TG(mRen2)27 rats on L-NAME treatment could be completely prevented by riociguat treatment at 10 mg/kg per day (riociguat high dose). The 5/6 nephrectomized riociguat-treated group (15 mg/kg per day) exhibited a significantly lower blood pressure compared to the untreated 5/6 nephrectomized sham-operated group at the end of the study.

3.1.2.4. Laboratory parameters

Rats of riociguat-treated groups had markedly lower plasma levels of creatinine and urea as well as reduced ANP levels in comparison to untreated controls for both observed hypertensive models.

In the 5/6 NX study part, creatinine clearance (CrCl) markedly decreased in both nephrectomized groups versus sham-operated controls during the time course of the experiment. Both uraemic groups started with the same CrCl, but at the end of study, the group treated with riociguat had a significantly higher CrCl than the untreated group.

3.1.2.5. Histology

In the high-renin study part, histopathological examination of the the heart and kidneys revealed that riociguat significantly reduced interstitial fibrosis and glomerulosclerosis compared to the L-NAME group.

In the low-renin study part, the diameter of cardiac myocytes of untreated nephrectomized rats was significantly increased compared to sham-operated controls. This effect was abolished in nephrectomized rats treated with riociguat. As expected, 5/6 NX induced a significant increase in renal interstitial fibrosis in both nephrectomized groups versus sham-operated control, but treatment with riociguat significantly suppressed this increase in nephrectomized animals.

3.2. SLV338 study

We examined the in vitro and in vivo features of SLV338 with special focus on the prevention of cardiac remodeling in an experimental model of renovascular hypertension (2-kidney, 1-clip [2K1C] model) in comparison with standard end organ protection with losartan.

3.2.1. In vitro selectivity profile of SLV338

In receptor binding experiments using cloned human receptors, SLV338 did not show relevant binding affinity (i.e. pKi values were > 5) towards a broad panel of receptors, including endothelin (ET_A, ET_B), angiotensin, bradykinin, chemokine, purinergic, adrenergic, muscarinic, nicotinic, dopaminergic, serotoninergic, histaminergic, glutamatergic, opioid, and a number of neuropeptide receptors and uptake sites. SLV338 did not show interaction with ion channels (L-type calcium channel, voltage-gated sodium channel, potassium channels).

The IC_{50} of SLV338 for inhibition of NEP was 7.9 nM; the IC_{50} of SLV338 for inhibition of ECE-1 is 5012 nM.

3.2.2. In vivo assessment of ECE/NEP-inhibition by SLV338.

Pretreatment with intravenous SLV338 5 min prior to the Big -ET-1 challenge potently and dose-dependently suppressed the pressor response. Half-maximal inhibition was reached approximately at a dose of 0.1 µmol/kg (= 0.06 mg/kg), with a minimally effective dose of 0.03 µmol/kg and higher. When the same experiments were carried out with SLV338 being administered orally 1h prior to big-ET challenge, the approximate ID_{50} was 30µmol/kg, which is about 17 mg/kg (data not shown).

Regarding functional NEP-inhibition by SLV338 in vivo, SLV338 induced a significant increase in ANP-stimulated urinary sodium- and cGMP-excretion and in plasma levels of ANP.

3.2.3. 2K1C renovascular hypertension and cardiac phenotype

3.2.3.1. Body and organ weight

At study end there was no difference in body weight between sham, 2K1C and 2K1C + SLV338 treatment (both dosages). There was a statistically significant increase in body weight in the 2K1C group treated with losartan versus both sham-operated and untreated 2K1C groups. However, this difference was less than 10% of body weight (versus sham controls). Regarding overall cardiac weight 2K1C controls exhibited a significantly increased heart weight versus sham controls. Treatment with SLV338 (high dose) and with losartan significantly reduced cardiac weight versus untreated 2K1C animals, while treatment with low dose SLV338 was accompanied with a non significant trend to lower values. The same pattern was observed with left ventricular weight. There was a significant decrease in weight of the clipped kidney and a significant increase in weight of the unclipped kidney in all 2K1C-groups vs. sham. There were no differences in kidney weight between the different treatment groups.

3.2.3.2. Blood pressure and heart rate

Two weeks after left kidney clipping, 2K1C rats developed severe hypertension compared with sham-operated controls. SLV338 had no impact on blood pressure in this model, in contrast to losartan, which reduced blood pressure by up to 46 mmHg, as compared to non-treated 2K1C controls. There was no difference in the heart rate between all study groups.

3.2.3.3. Cardiac histology

Interstitial fibrosis was markedly increased in the heart of 2K1C animals versus sham animals; treatment with both dosages of SLV338 and with losartan completely prevented this effect. The same pattern was observed with perivascular fibrosis. Media-lumen ratio of cardiac arteries was significantly elevated in non-treated 2K1C animals compared to sham controls. This effect was completely prevented by both doses of SLV338, and by losartan. Cardiomyocyte diameter was enlarged in non-treated 2K1C animals compared to sham-operated rats and this was completely suppressed by doses of both SLV338 and losartan.

Expression of active TGF-ß1 was assessed in the heart by immunohistochemical analysis. In sham-operated animals only weak staining of TGF-ß1 was observed in the myocardium, whereas in untreated 2K1C there was a marked and significant elevation of TGF-ß1 expression versus sham controls. Treatment with SLV 338 or losartan completely prevented elevation of TGF-ß1 expression in 2K1C animals.

3.3 SLV320 study

In this study, we investigated some in vitro and in vivo features of the selective adenosine A_1 receptor antagonist SLV 320 with special attention on the prevention of uremic cardiomyopathy in a rat model of chronic renal failure (5/6 NX).

3.3.1. In vitro selectivity profile of SLV320

SLV320 is a selective and potent adenosine A_1 antagonist in vitro (Ki = 1 nM) with a selectivity factor of at least 200 versus other adenosine receptor subtypes.

3.3.2. Haemodynamic effects of SLV320

Functional A1 antagonism could be confirmed in the rat: adenosine-induced bradycardia was prevented with ED_{50} values of 0.25 and 0.49 mg kg^{-1} after i.v. and oral application, respectively.

3.3.3. 5/6 nephrectomy and uraemic cardiomyopathy

3.3.3.1. Survival

After 5/6 NX was performed, only one rat in the 5/6 NX+SLV320 group died shortly before study end.

3.3.3.2. Body and organ weight

Mean body weight gain during the study period was 70 ± 1.5%, and there was no difference between the groups (Kruskal–Wallis, P=0.14). There was no significant difference between any of the groups in cardiac weights.

3.3.3.3. Blood pressure

Before 5/6 NX and treatment, there were no differences in blood pressure between the study groups. In week 1, the 5/6 NX+SLV320 group exhibited a significantly higher blood pressure compared to the untreated 5/6 NX group, whereas for the remainder of the study no differences between the two nephrectomized groups were detected.

3.3.3.4. Plasma and urinary parameters and GFR

GFR significantly decreased in both nephrectomized groups versus sham controls; no effects of treatment with SLV320 on healthy or nephrectomized animals were detected. The same pattern was present when plasma urea and creatinine levels were assessed. When urinary albumin excretion was

assessed, no difference was found between the non-treated and SLV320-treated sham-operated groups. Both nephrectomized groups exhibited a significantly increased urinary albumin excretion compared to sham animals. However, this albuminuria was significantly attenuated by about 50% by treatment with SLV320.

The plasma levels of CK, ALT and AST did not differ between the sham-operated groups. However, there was a trend to higher CK levels and a significant increase in ALT and AST plasma levels in untreated nephrectomized animals when compared to sham animals. Treatment with SLV320 significantly suppressed this increase in nephrectomized animals.

Plasma levels of albumin were significantly lower in untreated nephrectomized animals versus sham-operated animals, while nephrectomized animals treated with SLV320 did not differ from sham controls.

3.3.3.5. Cardiac and renal histology

Regarding heart weight, media/lumen ratio of cardiac arteries and perivascular fibrosis, no differences were detected between the study groups. Immunohistochemistry revealed a significant increase of collagen I and III in untreated nephrectomized rats when compared to sham-operated animals. However, this effect was totally suppressed in nephrectomized animals treated with SLV320.

In the kidney, no effect of treatment with SLV320 on fibrosis was detected.

4. Discussion

4.1. Cardio-renal effects of riociguat

The study is the first to analyze the cardiovascular and renal consequences of direct stimulation of sGC by the novel stimulator of sGC riociguat in low-renin and high-renin rat models of hypertension.

Despite the widespread use of organic nitrates in the treatment of cardiovascular diseases, the development of tolerance upon prolonged use limits the therapeutic value of this class of compounds for chronic treatment (33, 34). Therefore, compounds which can stimulate sGC in a NO-independent manner may have therapeutic advantages over traditional nitrovasodilators. The vasorelaxant effect of riociguat was resistant to the development of tolerance. Vasorelaxation caused by riociguat in aortic rings taken from normal and nitrate-tolerant rabbits was almost the same. These observations indicate that riociguat may offer considerable advantages by overcoming the tolerance developed during sustained traditional nitrovasodilator therapy.

NO, via activation of the sGC/cGMP signaling pathway, modulates hypertension in different species and attenuates the hypertrophic response to growth factor stimulation in cardiomyocytes (35, 36). Endothelial NO deficiency and dysfunctional sGC, in turn, promote hypertension and accelerate progression of renal disease (37, 38). We, therefore, hypothesized that direct sGC stimulation by riociguat may attenuate hypertension in an NO-independent manner and reduce the cardiac and renal damage caused by raising the intracellular cGMP.

The cardio-renal effects of riociguat were evaluated in two independent experimental rat models of systemic hypertension: the TG(mRen2)27 rats treated with L-NAME (high-renin model) and rats with 5/6 nephrectomy (low-renin model). These models were chosen because they represent different pathways leading to hypertension and the degree of hypertension is different as well. L-NAME administration on top of transgenic overexpression of renin was used to show that the observed effects were nitric oxide-independent and based solely on the action of riociguat in a high-renin malignant model of hypertension.

Riociguat did not exhibit any cardiotoxicity, nephrotoxicity or hepatotoxicity in the current studies, supporting previous work in experimental models (4) and clinical studies (32, 39, 40).

In both animal models, riociguat reversed the increase in blood pressure by completely normalizing the pressure values in L-NAME-treated TG(mRen2)27 rats by cotreatment with riociguat (10 mg/kg). Similar results were obtained for the sGC stimulator BAY 41-2272, which completely prevented the increase in systolic blood pressure without any signs of tolerance in L-NAME-treated TG(mRen2)27 rats (41, 42).

ANP is released in response to increased wall tension (43), (44) and has a powerful diagnostic and prognostic value in patients with hypertension and chronic renal failure (45, 46). In endothelial cells, cGMP is synthesized by two distinct guanylate cyclase (GC) isoforms, the NO-activated sGC and the ANP-activated particulate GC (GC-A) (47, 48). Since the ANP-induced particulate GS activity may be questioned during stimulation of sGC by riociguat, ANP levels were measured in the study groups. The high plasma level of ANP in both animal models may reflect cardiac functional and structural damage. The plasma level of ANP was significantly lower in animals treated with riociguat compared to untreated animals suggesting cardiorenal protective effects of this compound.

Riociguat attenuated ventricular hypertrophy in both animal models compared with the untreated uraemic groups. Histomorphometric analysis revealed a strong myocyte diameter reduction in 5/6 nephrectomized rats treated with riociguat and reducing cardiac fibrosis observed in riociguat-treated TG(mRen2)27 rats compared to untreated animals. Regarding renoprotective effects of sGC stimulation, riociguat suppressed the increase in urea and creatinine in both models of hypertension and improved creatinine clearance after 5/6 nephrectomy. Riociguat prevented kidney tissue

damage as indicated by the compound's capacity to abolish L-NAME-induced glomerulosclerosis and interstitial fibrosis in both models. Even though we did not know whether the cardio-renal effects of riociguat were due to sGC stimulation or simply to blood pressure reduction, it is evident that the natriuretic peptides–particulate GC system suppresses cardiac hypertrophy and fibrosis independently of blood pressure (49, 50, 51). The anti-remodelling effect of riociguat was demonstrated in two independent animal models of pulmonary hypertension (4). Moreover, in our study, the attenuation of cardiac and renal fibrosis in riociguat-treated animals is more likely a consequence of notable reduction in collagen deposition in cardiac and kidney tissue. This concept is supported by several other recent studies documenting antifibrotic effect of sGC stimulator BAY 41-2272 in a progressive model of anti-thy1-induced chronic glomerulosclerosis (52, 53).

In sum, our study represents the first pharmacological evidence that the novel sGC stimulator riociguat confers potent protection against cardiac and renal end-organ damage in two independent rat models of high-renin and low-renin hypertension.

4.2. SLV 338 study

This study is the first to demonstrate blood pressure–independent cardioprotective effects of ECE/NEP inhibition on cardiac remodeling, as well as coronary vascular adaptaion in a rat model of renovascular hypertension.

Interestingly, cardioprotective action of SLV338 was comparable to that of losartan. This observation is remarkable on two accounts: systemic pathological changes occurring in this renovascular model of hypertension are largely driven by the activation of the renin-angiotensin system, and at least part of the cardiac remodeling is considered to be secondary to the hypertension that results from the renal artery clipping (54). Therefore, the end organ protection provided by the AT_1 receptor antagonists, blocking angiotensin II (ANG II) effects via the AT_1 receptor, was expected but less so the effects of SLV338, which does not primarily target the renin-angiotensin system and had no antihypertensive activity at all in 2K1C model. Blood pressure in the 2K1C rat does not respond to ET receptor antagonists, so that the 2K1C model may be considered an "endothelin-1-independent" hypertensive model (55).

These data are in agreement with another study using selective endothelin receptor A (ETA) and ETB receptor antagonists in the same animal model (12). There it was demonstrated that an overall activation of the cardiac endothelin system was not detectable in the 2K1C rat, which does not exclude an activated endothelin system in specific cell types, for example, cardiac fibroblasts. The authors concluded that ETA antagonism reduced vascular remodelling, whereas ETB antagonism reduced fibrosis, which supports the findings in our present study.

ET-1 has been reported to mediate some of the effects of ANG II (downstream of the AT_1 receptors) (56, 57, 58). If this is true, one would expect that both drugs act via the same downstream effector pathways. To further clarify this point we evaluated $TGF\beta_1$ expression in the heart tissues. TGF-β1 is one of the major profibrogenic cytokines associated with organ fibrosis. This cytokine has been shown to be activated by both RAS and endothelin system (59). Cardiac $TGF\beta_1$ expression was remarkably higher in untreated 2K1C rats compared to sham-operated rats. Overexpression was normalized by treatment with either SLV 338 or losartan. These findings support the hypothesis that both drugs might be involved in the same pathway. Moreover, it is known that ET-1, ANG II, and TGF-β1 are functionally associated with connective tissue growth factor and platelet-derived growth factor, forming a signaling network that is vital both in normal tissue repair and maladaptive fibrotic remodeling (60). Future studies targeting all of the components of this network singly or in combination are required to optimize therapeutic approach in maladaptive cardiac remodeling.

A limitation of our study is that we did not include sole NEP inhibition on cardiac target organ protection. In experimental models of salt-sensitive hypertension (61) and post-infarction heart failure (62), combined ECE /NEP inhibition led to the improvement of cardiac function and to myocardial remodelling. A study by Tikkanen et al. (63) also showed significant blood pressure reduction by ECE/NEP inhibition in diabetic rats and concomitant reduction in heart:body weight ratio. However, in none of the above cited studies dealing with ECE/NEP inhibition was there a significant beneficial impact of sole NEP inhibition on cardiac target organ protection.

Taken together, the dual ECE/NEP inhibitor SLV338 prevents cardiac remodeling in a rat model of renovascular hypertension to the same extent as losartan, but in a blood pressure–independent manner. This effect is at least partially mediated via suppression of the cardiac TGF-β1 expression.

4.3. SLV320 study

This study has revealed for the first time that blockade of the adenosine A1 receptor had protective effects in the heart and kidney by attenuating cardiac fibrosis and albuminuria in rats with 5/6 NX and that these protective effects occurred without changes in blood pressure. Our immunohistochemical observation results demonstrate that administration of SLV320 in nephrectomized animals lowered the amount of collagen I and III to baseline levels. The occurrence of cardiac fibrosis in our model is in agreement with two previous studies which concluded that uremia promotes cardiac fibrosis independently of hypertension (64, 65)

In line with this finding are data indicating that cardiac overexpression of the A1 adenosine receptor in mice causes myocardial fibrosis associated with an increased mortality (66). We suggest that part of the cardioprotective effect due to antagonism of the A_1 receptor is mediated by blockade of A_1

receptors on neutrophils, leading to a reduction in the neutrophil chemoattractant response (15, 16; 17).

An alternative explanation for our findings is that blockade of the cardiac A1 adenosine receptors may increase the action of adenosine on A2 receptors. It is well known that adenosine exerts inhibitory effects on cardiac fibroblast proliferation and matrix synthesis via A2 receptors (67, 68, 69). Thus, the observed effects may possibly be attributed to enhanced agonist activity on cardiac A2 receptors by endogenous adenosine as a consequence of blocking the A1 receptors by SLV320.

Prior observations indicate that risk factors related to kidney function correlate much better with outcome compared with classical cardiac risk factors in patients with chronic heart failure (70). Treatment with SLV320 led to a 50% reduction in albumin excretion compared to untreated 5/6 nephrectomized rats. Treatment with SLV320 did not significantly lower creatinine or increase GFR. As albuminuria is an early and sensitive marker of renal and cardiovascular risk (71), we conclude that treatment with SLV320 does indeed exert beneficial effects on renal disease progression, but long-term studies are needed to prove, whether this effect is translated into better preservation of GFR.

In conclusion, these findings indicate the potential usefulness of a novel A1 receptor antagonist, SLV320, in the amelioration of cardiac fibrosis and albuminuria in rats with 5/6 NX without affecting blood pressure.

5. Conclusions

We demonstrated both blood-pressure-dependent and blood-pressure-independent approaches for the treatment of hypertension-induced target organ damage. The sGC stimulator riociguat in particular seems to be promising under conditions of malignant hypertension where other drugs may be less effective. On the other hand, the application of the combined ECE/NEP inhibitor SLV 338 as well as the novel A1 receptor antagonist SLV320 provides novel pharmacological strategies for preventing hypertension-induced end-organ damage independent of blood-pressure lowering effects. In the future, these approaches will have to be balanced with the established beneficial effects of current antihypertensive therapies.

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Anteilserklärung

Yuliya Sharkovska hatte folgenden Anteil an den vorgelegten Publikationen

1. Kalk P, Eggert B, Relle K, Godes M, Heiden S, **Sharkovska Y**, Fischer Y, Ziegler D, Bielenberg GW, Hocher B. The adenosine A1 receptor antagonist SLV320 reduces myocardial fibrosis in rats with 5/6 nephrectomy without affecting blood pressure.
Br J Pharmacol. 2007 Aug;151(7):1025-32.
Impact Factor: 3.767
Anteil: 20%
Beitrag im Einzelnen:
Beteiligung an Histologische Auswertung der Proben, Bearbeitung der Ergebnisse, Erstellung des Bildmaterials, Überarbeitung des Manuskripts.

2. **Sharkovska Y**, Kalk P, Lawrenz B, Godes M, Hoffmann S, Wellkisch K, Geschka S, Relle K, Hocher B, Stasch J. NO-independent Stimulation of Soluble Guanylate Cyclase reduces target organ damage in low- and high-renin models of hypertension.
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Beteiligung an Idee, Konzept und Design der Studie, Aufbereitung der Proben, Auswahl der Testverfahren, Beteiligung an Histologische Auswertung der Proben, Bearbeitung der Ergebnisse, Erstellung des Bildmaterials, Überarbeitung des Manuskripts.

3. Kalk P*, **Sharkovska Y***,Kashina E, von Websky K, Relle K, Pfab T, Alter M, Guillaume P, Provost D, Hoffmann K, Fischer Y, Hocher B The Combined Endothelin-Converting Enzyme / Neutral Endopeptidase Inhibitor SLV338 Prevents Myocardial Remodelling in Rats with Renovascular Hypertension in a Blood Pressure Independent Manner.
Hypertension. 2011 Apr;57(4):755-63.
*: both authors contributed equally
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Beitrag im Einzelnen:
Beteiligung an Idee, Konzept und Design der Studie, Aufbereitung der Proben, Auswahl der Testverfahren, Beteiligung an Histologische/Immunohistologische Auswertung der Proben, Bearbeitung der Ergebnisse, Erstellung des Bildmaterials, Überarbeitung des Manuskripts.

Yuliya Sharkovska (Dokrorandin)

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Complete list of publications

1. The novel DPP-4 inhibitors linagliptin and BI 14361 reduce infarct size after myocardial ischemia/reperfusion in rats. Hocher B*, Sharkovska Y*, Mark M, Klein T, Pfab T. Int J Cardiol. 2012
   *: both authors contributed equally
2. Renoprotective Effects of Combined Endothelin-Converting Enzyme / Neutral Endopeptidase Inhibitor SLV338 in Acute and Chronic Experimental Renal Damage Clinical Laboratory. Sharkovska Y, Kalk P, von Websky K, Relle K, Pfab T, Alter M, Fischer Y, Hocher B. - Clin Lab. 2011;57(7-8):507-15
3. Soluble guanylate cyclase stimulation prevents fibrotic tissue remodeling and improves survival in salt-sensitive dahl rats. Geschka S, Kretschmer A, Sharkovska Y, Evgenov OV, Lawrenz B, Hucke A, Hocher B, Stasch JP. - PLoS One. 2011;6(7):e21853.
4. The Combined Endothelin-Converting Enzyme / Neutral Endopeptidase Inhibitor SLV338 Prevents Myocardial Remodelling in Rats with Renovascular Hypertension in a Blood Pressure Independent Manner. Kalk P*, Sharkovska Y*, Kashina E, von Websky K, Relle K, Pfab T, Alter M, Guillaume P, Provost D, Hoffmann K, Fischer Y, Hocher B. – Hypertension. 2011 Apr;57(4):755-63. Epub 2011 Feb 21.
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5. Endothelin-1 overexpression restores diastolic function in eNOS knockout mice. Vignon-Zellweger N, Relle K, Kienlen E, Alter M, Seider P, Sharkovska J, Heiden S, Kalk P, Schwab K, Albrecht-Küpper B, Theuring F, Stasch JP, Hocher B.
6. NO-independent Stimulation of Soluble Guanylate Cyclase reduces target organ damage in low- and high-renin models of hypertension. Sharkovska Y and Kalk P, Lawrenz B, Godes M, Hoffmann S, Wellkisch K, Geschka S, Relle K, Hocher B, Stasch J. – J Hypertens. 2010 Aug; - 28(8):1666-75.
7. Post-obstructive regeneration of the kidney is derailed, when surge in renal stem cells during the course of unilateral ureteral obstruction is halted. Park H, Yasuda K, Ratliff B, Stoessel A, Sharkovska Y, Yamamoto I, Jasmin J, Bachmann S, Lisanti L, Chander P, and Goligorsky M. AJP - Am J Physiol Renal Physiol. 2009 Nov 11.
8. Pulmonary fibrosis in L-NAME-treated mice is dependent on an activated endothelin system. Kalk P, Mach A, Thone-Reineke C, Godes M, Heiden S, Sharkovska Y, von Websky K, Relle K, Hocher B. Can J Physiol Pharmacol. 2008 Aug;86(8):541-5.
9. The adenosine A1 receptor antagonist SLV320 reduces myocardial fibrosis in rats with 5/6 nephrectomy without affecting blood pressure. Kalk P, Eggert B, Relle K, Godes M, Heiden S, Sharkovska Y, Fischer Y, Ziegler D, Bielenberg GW, Hocher B. Br J Pharmacol. 2007 Aug;151(7):1025-32.
10. The diagnostic method of the activity of pyelonephritis in children. Odynetz Y, Sharkovska Y. Patent 28610 UA (Application number : u200711042).
11. The indices of neurohumoral regulation in children suffering from pyelonephritis. Sharkovska Y. European J of Medical Research; 17th European students' conference: Materials of conference, Berlin, Germany. 2006:103
12. The characteristics of central and renal hemodynamics in children suffering from acute and chronic pyelonephritis. Sharkovska Y and Odynetz Y; 16th European students' conference: Materials of conference, Berlin, Germany. 2005.

Erklärung

„Ich, Sharkovska Yuliya, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: „New approaches in the treatment of target organ damage in experimental hypertension“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

Datum 01.02.2012 Yuliya Sharkovska

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