Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 520 (2005) 179–187

www.elsevier.com/locate/ejphar

Protection against oxidative stress in diabetic rats: Role of angiotensin AT_1 receptor and beta 1-adrenoceptor antagonism

Marc Dorenkamp [a,1], Alexander Riad [a,1], Sebastian Stiehl [b], Frank Spillmann [a], Dirk Westermann [a], Jing Du [a], Matthias Pauschinger [a], Michel Noutsias [a], Volker Adams [b], Heinz-Peter Schultheiss [a], Carsten Tschöpe [a,*]

[a] *Department of Cardiology and Pneumonology, Charité - Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany*

[b] *University Leipzig, Heart Center Leipzig, Clinic of Cardiology, Germany*

Received 7 February 2005; received in revised form 19 May 2005; accepted 19 July 2005
Available online 31 August 2005

Abstract

Oxidative stress and low-grade inflammation are hallmarks of diabetes mellitus. We explored protective, blood pressure-independent effects of the angiotensin II type 1 (AT_1) receptor antagonist candesartan and the selective β_1-adrenoceptor antagonist metoprolol. Diabetes mellitus was induced in 8-week-old Sprague–Dawley rats after injection of streptozotocin. Diabetic rats were randomized to treatment with candesartan or metoprolol in sub-antihypertensive doses or to placebo treatment. In the quadriceps, musculature markers of oxidative stress and inflammation were determined. Function of the inherent vascular bed was measured in vivo in the autoperfused hindlimb. Increases in NAD(P)H activity, expression of its cytosolic subunit $p22^{phox}$ and of endothelial NO synthase e(NOS) displayed enhanced oxidative stress. Upregulated intercellular (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 and of inducible NOS (iNOS) revealed inflammatory processes. Diabetes was associated with severe impairment of endothelium-dependent and -independent vasodilatation. Candesartan, but not metoprolol, reduced NAD(P)H activity, attenuated diabetes-induced over-expression of $p22^{phox}$ and eNOS mRNA as well as ICAM-1, VCAM-1, iNOS and eNOS immunoreactivity and led to a substantial improvement of endothelium-dependent vasodilatation (+46.3% vs. placebo treatment; $P<0.05$). Angiotensin AT_1 receptor antagonism, but not β_1-adrenoceptor antagonism, ameliorates diabetes-generated oxidative stress, indicating a pivotal role of the renin–angiotensin system in the development of diabetic complications.

Keywords: Diabetes mellitus; Endothelial dysfunction; NAD(P)H oxidase; Oxygen radical; Beta 1-adrenoceptor antagonist; Angiotensin AT_1 receptor antagonist

1. Introduction

There is definite evidence that reactive oxygen species contribute to the progression of diabetes mellitus, its complications, associated cardiovascular disease and atherosclerosis (Evans et al., 2002). Enhanced oxidative stress reduces availability of nitric oxide (NO) and promotes redox-sensitive expression of adhesion molecules like intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (Hsueh and Quinones, 2003). These cell surface glycoproteins facilitate the attachment of blood-borne leukocytes to the endothelium and are crucial for the resulting low-grade inflammation (Harrison, 1997). Angiotensin II accelerates the development of diabetic complications by promoting the generation of superoxide anions by activation of the angiotensin AT_1 receptor (Rajagopalan et al., 1996). The angiotensin AT_1 receptor also mediates synthesis of pro-inflammatory cytokines and adhesion molecules (Tummala et al., 1999). Recent studies suggest benefits of angiotensin AT_1

* Corresponding author. Tel.: +49 30 8445 2349; fax: +49 30 8445 4648.
E-mail address: ctschoepe@yahoo.com (C. Tschöpe).
[1] Both authors contributed equally.

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.07.020

antagonists beyond a reduction in blood pressure (Lindholm et al., 2002). In this regard, angiotensin AT_1 antagonists could diminish the production of superoxide anions, leading to protective effects in diabetics. However, in recent clinical trials, various β-adrenoceptor antagonists have been proven to be also of prognostic benefit in diabetic patients (Haas et al., 2003). This finding gives rise to the hypotheses regarding the mechanisms underlying the beneficial effect of β-adrenoceptor antagonists in diabetic subjects. This study was performed to compare blood-pressure-independent effects of the angiotensin AT_1 receptor antagonist candesartan and the selective β_1-adrenoceptor antagonist metoprolol on oxidative stress in streptozotocin-induced diabetic rats. To elucidate pathways mediating protective effects, the expression of several genes and proteins involved in generation of oxidative stress and inflammatory cascades were investigated in the quadriceps musculature and as a functional aspect the inherent vascular bed was characterized in vivo in the autoperfused hindlimb model.

2. Material and methods

2.1. Experimental animals

Experiments were performed in 8-week-old male Sprague–Dawley rats weighing 300–320 g (Charles River, Berlin, Germany). Rats were housed under standard conditions (20 °C, 12-h light/dark cycle) and given free access to water and standard food. The investigation conforms with 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). Diabetes mellitus was induced by a single intraperitoneal injection of 70 mg/kg of streptozotocin (Sigma, St. Louis, USA) dissolved in 0.1 mol/l citric acid–trisodium citrate buffer with a pH of 4.5 as previously described (Tschöpe et al., 1999). Control rats ($n=7$) received citrate buffer alone. After 7, 14, 21 and 48 days, blood glucose concentrations were measured using a glucometer (Accutrend sensor, Boehringer Mannheim, Germany). All animals were weighted weekly. Non-diabetic Sprague–Dawley rats served as controls (Group 1) ($n=7$). Streptozotocin-diabetic animals were further randomised to receive vehicle (10% β-cyclodextrane solution) (Group 2), the angiotensin AT_1 receptor antagonist candesartan (Astra Zeneca, Wedel, Germany) (1.5 mg/kg body weight per day by gavage) (Group 3) and the β_1-adrenoceptor antagonist metoprolol (Astra Zeneca, Wedel, Germany) (30 mg/kg body weight per day by gavage) (Group 4), using sub-antihypertensive doses, respectively ($n=8$ each group). Treatment was started 5 days after induction of diabetes and was continued for 48 days.

2.2. Hindlimb perfusion and blood pressure measurement

For the evaluation of vascular function, the autoperfused hindlimb model was taken as described before (Angulo et al., 1998). Briefly, on treatment day 48 the rats were anaesthetized with chloral hydrate (Sigma, St. Louis, USA) (400 mg/kg, i.p.). After intubation, the animals were mechanically ventilated with a respirator (Ugo Basile, Comerio, Italy) and their temperature was maintained at 37 °C with a thermic table. Under aseptic conditions, catheters (PP20) were placed in both carotid arteries. Systemic blood pressure and heart rate were recorded from the right carotid artery through a tip-catheter (2F) system (Millar, Föhr Medical Instruments, Seeheim, Germany). A femoral artery catheter was placed through a separate incision into the right groin. Perfusion of the left hindlimb was performed by means of a roller peristaltic pump (Minipuls 3, Abimed, Langenfeld, Germany), which delivered blood from the left carotid artery. Flow was set at 2.2 ml/min per kilogram bodyweight. The hindlimb perfusion pressure was recorded with a pressure transducer (Medex Inc., Carlsbad, USA) located distal to the peristaltic pump. Extracorporal volume was 1.1 ml, filled with Krebs–Henseleit solution containing sodium heparin (60 IE/ml). The flow rate was kept constant so that basal vascular resistance to flow could be compared between experimental groups. An infusion pump was connected on the cannula side of the peristaltic pump. This allowed us to obtain depressor responses to infusion of vasoactive drugs and to flow-mediated vasodilatation by infusion of Krebs–Henseleit solution.

2.3. Endothelium-dependent and -independent vasodilatation

Infusion of Krebs–Henseleit solution was used to estimate flow-mediated endothelium-dependent vasorelaxation. In order to induce graduated flow-dependent vasodilatation, three different volumes of Krebs–Henseleit solution (80, 200 and 600 μl/kg) were administered. The response elicited by each dose of Krebs–Henseleit solution was obtained from the baseline vasopressor tone, the effect of any previous infusion having entirely disappeared. When circulatory parameters were again stabilized, sodium nitroprusside (Sigma, St. Louis, USA) (40 μg/kg) was used to estimate vascular responsiveness to exogenous NO (Angulo et al., 1998). The rats were anaesthetized throughout the entire measurements.

2.4. Real-time reverse transcription-polymerase chain reaction

Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed using the Light Cycler system (Roche, Mannheim, Germany). Total RNA was isolated from each quadriceps muscle and cDNA was