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Improved endothelial function and reduced platelet activation by chronic HMG-CoA-reductase inhibition with rosuvastatin in rats with streptozotocin-induced diabetes mellitus

Andreas Schäfer [a,*], *Daniela Fraccarollo* [a], *Christian Vogt* [a], *Ulrike Flierl* [a], *Melinda Hemberger* [a], *Piet Tas* [b], *Georg Ertl* [a], *Johann Bauersachs* [a]

[a] *Medizinische Klinik und Poliklinik I, Universitätsklinikum Würzburg, Universität Würzburg, Germany*
[b] *Klinik und Poliklinik für Anästhesiologie, Universitätsklinikum Würzburg, Germany*

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ABSTRACT

Diabetes is associated with endothelial dysfunction and platelet activation, both of which may contribute to increased cardiovascular risk. We investigated whether the hydroxy-3-methyl-glutaryl CoA reductase inhibitor rosuvastatin improves endothelial function and reduces platelet activation in diabetic rats. Therefore, male Wistar rats were injected with streptozotocin (STZ, 50 mg/kg i.v.) to induce insulin-deficient diabetes. Treatment with rosuvastatin (20 mg/[kg day]) or vehicle was initiated 2 weeks after injection of STZ and continued for 2 weeks. Thereafter, platelet activation was assessed in fresh whole blood and vascular function was characterized in isolated aortic segments in organ bath chambers. Endothelium-dependent relaxation induced by acetylcholine was significantly attenuated in diabetic rats and improved by treatment with rosuvastatin (maximum relaxation, % of precontraction—control: 99.8 ± 0.2, STZ-vehicle: 80.7 ± 2.9, STZ-rosuvastatin: 98.9 ± 0.7; $p < 0.01$). Similarly, treatment with rosuvastatin significantly reduced fibrinogen-binding to activated GPIIb/IIIa (mean fluorescence—control: 161.0 ± 6.9, STZ-vehicle: 207.8 ± 15.9, rosuvastatin: 173.6 ± 5.3; $p < 0.05$) and P-Selectin surface expression on platelets (mean fluorescence—control: 76.5 ± 7.3, STZ-vehicle: 92.1 ± 5.5, rosuvastatin: 75.2 ± 6.5; $p < 0.05$), while both markers of platelet activation were increased in diabetic rats. Therefore, rosuvastatin treatment normalizes endothelial function and reduces platelet activation in diabetic rats. These effects may contribute to the reduction of cardiovascular events by statins in diabetic patients.

1. Introduction

Diabetes is strongly associated with cardiovascular disease, which is the primary cause of morbidity and mortality among patients with diabetes, accounting for more than 80% of deaths [1]. Diabetes alone confers long-term cardiovascular risk similar to that observed among non-diabetic patients with prior myocardial infarction [2]. Patients with diabetes have early development of abnormal endothelial function, platelet hyper-reactivity,

* *Corresponding author at:* Medizinische Klinik und Poliklinik I, Universitätsklinikum Würzburg, Josef-Schneider-Str. 2, 97080 Würzburg, Germany. Tel.: +49 931 201 1; fax: +49 931 201 36131.
E-mail address: a.schaefer@medizin.uni-wuerzburg.de (A. Schäfer).
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aggressive atherosclerosis, and adverse arterial remodelling [1].

The risk for cardiovascular disease is already substantially elevated before diagnosis of diabetes [3] and progression of atherosclerosis is accelerated in diabetics [4]. Reduced nitric oxide (NO) bioavailability and abundant formation of reactive oxygen species (ROS) within the vascular wall are the key determinants in endothelial dysfunction resulting in an imbalance between NO and ROS. Impaired endothelial function has been described in very early stages of diabetes mellitus and hyperglycemia, and decreased insulin-sensitivity, as well as increased oxidative stress, have been proposed as possible contributors (as reviewed by [5]).

Platelet activation occurs in several cardiovascular diseases with reduced NO bioavailability, such as acute coronary syndrome [6], heart failure [7–9], insulin resistance [10], and diabetes [11]. Platelet activation leads to shape change, degranulation and rapid surface-expression of adhesion molecules such as P-selectin [12], which strongly participates in platelet adhesion to leukocytes [13,14]. Activated platelets are essential for promoting leukocyte adhesion and determining the progression of atherosclerotic lesion formation [15]. Moreover, platelet activation plays a critical role in the initiation of atherosclerosis, as demonstrated in a model of accelerated atherosclerosis in mice, in which inhibition of activated platelets using glycoprotein IIb/IIIa inhibitors prevented the development of atherosclerotic lesions [15].

We recently demonstrated that acute [16] and chronic [17] reduction of systemic NO bioavailability results in platelet activation in vivo. In addition to its effects on vascular tone, NO is a central regulator of platelet activation, adhesion and aggregation: reduced NO bioactivity is associated with arterial thrombosis in animal models and in individuals with endothelial dysfunction [18]. We also demonstrated that normalization of systemic NO bioavailability in diabetes increases endogenous platelet inhibition by an NO/cGMP-mediated signaling pathway resulting in reduced platelet activation [17]. These results underline the critical role of systemic NO bioavailability for regulation/inhibition of platelet activation.

Hydroxy-3-methyl-glutaryl (HMG)-CoA-reductase inhibitors are recommended as first-line therapy for treatment of dyslipidemia in diabetic patients [19], and rosuvastatin has previously been demonstrated to be effective in diabetic patients [20]. We examined the effects of chronic HMG-CoA-reductase inhibition by rosuvastatin on vascular endothelial function and platelet activation in streptozotocin-induced diabetes in rats.

2. Materials and methods

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).

2.1. Animals

Male Wistar rats (250–300 g, obtained from Harlan-Winkelmann, Borchen, Germany) were housed in temperature-controlled cages (20–22 °C) with a 12-h light–dark cycle, and given free access to water and formulated diets.

2.2. Induction of diabetes by streptozotocin injection

A single dose streptozotocin (STZ) regimen was used to induce pancreatic islet cell destruction and persistent hyperglycaemia. STZ (10 mg/ml, Sigma, Deisenhofen, Germany) was freshly dissolved in sterile sodium citrate buffer (25 mmol/l, pH 4.5) and used within 10 min. Rats received a single 50 mg/kg intravenous injection of STZ or citrate buffer (control). Blood glucose was monitored using a one-touch blood glucose meter (Ascensea Elite, Bayer-Vital GmbH, Leverkusen, Germany). Hyperglycaemia was defined as a random blood glucose level >20 mmol/l at 2 and 4 weeks after injection. Rats were randomized to placebo or rosuvastatin (20 mg/kg/day, AstraZeneca, which is the 90% HMG-CoA-reductase inhibiting dose in rats [21]) at day 14. Two weeks later, vasomotor function and platelet activation were assessed. Since Rosuvastatin did neither influence vascular function nor platelet activation after 12 weeks of treatment in non-diabetic Wistar rats in a previous study (data not shown), we did not include such a group in the present study.

2.3. Platelet sampling and aortic harvesting

Deep general anesthesia was induced using isoflurane. The abdominal cavity was opened under deep anesthesia, determined by total absence of reaction to pain under spontaneous respiration, and blood was taken by direct puncture of the inferior caval vein into a tube containing 3.8% sodium citrate. After exanguination the descending thoracic aorta was dissected following removal of the heart and cleaned of connective tissue. One section was used for measurement of O_2^- production, while the other was cut into 3 mm rings which were mounted in an organ bath (FMI, Seeheim, Germany) for isometric force measurements.

2.4. Vascular reactivity studies

The rings were equilibrated for 30 min under a resting tension of 2 g in oxygenated (95% O_2; 5% CO_2) Krebs–Henseleit solution (NaCl 118 mmol/l, KCl 4.7 mmol/l, $MgSO_4$ 1.2 mmol/l, $CaCl_2$ 1.6 mmol/l, KH_2PO_4 1.2 mmol/l, $NaHCO_3$ 25 mmol/l, glucose 12 mmol/l; pH 7.4, 37 °C) containing diclofenac (1 μmol/l). Rings were repeatedly contracted by KCl (with a maximum of 100 mmol/l) until reproducible responses were obtained.

The relaxant response to cumulative concentrations of acetylcholine was assessed after preconstriction with phenylephrine to comparable levels. Following repetitive washouts and resting periods for at least 30 min, aortic rings were slightly preconstricted to about 20% of the maximal constriction with low, incremental doses of phenylephrine and the additional contraction to L-NNA was measured as a marker of physiological stretch-induced, calcium-independent NO formation. Furthermore, relaxant responses to the endothelium-independent vasodilator 2-(*N*,*N*-diethylamino)-diazenolate-2-oxide (DEA-NONOate, Alexis Biochemicals, San Diego, CA) were determined after preconstriction with phenylephrine in the presence of L-NNA.

2.5. Measurement of superoxide anion formation

Vascular O_2^- formation was measured using lucigenin-enhanced chemiluminescence. The light reaction between O_2^- and lucigenin (5 μmol/l) was detected in a luminometer (Wallac, Freiburg, Germany) during incubation of rings in a HEPES-modified Krebs buffer (pH 7.40).

The oxidative fluorescent dye hydroethidine was used to evaluate in situ production of superoxide as previously described. Unfixed frozen ring segments were cut into 10-μm-thick sections and placed on a glass slide. Hydroethidine (2 μmol/l) was topically applied to each tissue section and coverslipped. Slides were incubated in a light-protected humidified chamber at 37 °C for 30 min. Images were obtained with a Bio-Rad MRC-1024 laser scanning confocal microscope equipped with a krypton/argon laser. Aortic rings from diabetic animals and control tissues were processed and imaged in parallel. Laser settings were identical for acquisition of images from diabetic and control specimens. Fluorescence was detected with a 585-nm long-pass filter. Quantitative analysis of hydroxyethidium fluorescence was performed using NIH ImageJ.

2.6. Flow cytometry

Whole blood was diluted with PBS (free of Ca^{2+} and Mg^{2+}, enriched with D-glucose [5.5 mmol/l] and 0.5% BSA). Platelet-bound fibrinogen was determined by incubation with a FITC-labeled anti-fibrinogen antibody (WAK-Chemie, Bad Soden, Germany) [22] for 10 min. For determination of surface-expressed P-selectin, diluted blood was incubated with a polyclonal rabbit anti-P-selectin (CD62P) antibody (Becton Dickinson, Heidelberg, Germany) for 10 min at room temperature followed by incubation with a FITC-labeled goat anti-rabbit IgG-antibody (Jackson ImmunoResearch, West Grove, Pennsylvania). Staining of the samples was also performed only with the FITC-conjugated secondary antibody in the absence of the primary antibody and served as negative control samples. An anti-rat CD42 (glycoprotein Ib-V-IX complex) monoclonal FITC-conjugated antibody (Becton Dickinson) was used as a platelet-specific marker for detection of circulating platelet-derived microparticles (PMP). Platelet CD42 expression was significantly higher in control versus diabetic platelets, which precluded the inclusion of healthy controls into the evaluation of PMPs. CD42 expression, however, was not significantly different between the two diabetic groups (data not shown). Following incubation with the antibodies, platelets were fixed with methanol-free formaldehyde (1.5%) for 10 min, and subsequently analyzed in a Becton Dickinson FACSCalibur at a low flow rate. The platelet population was identified on its forward and side scatter distribution, and 20,000 events were analyzed for mean fluorescence using CELLQuest software, Version 3.1f; non-specific binding was arbitrarily adjusted to a mean fluorescence of 10 and visually subtracted in the graphs.

For detection of PMP, all events in a whole blood sample were acquired until 20,000 events had been counted within a platelet gate. Microparticles were characterized by forward- and sideward-scatter of less than 10 and being outside any defined cell population. PMP were defined as the $CD42^+$ events within this microparticle region and the amount of PMP was expressed as $CD42^+$ microparticles/all $CD42^+$ events.

2.7. Western blot analysis

Aorta samples were homogenized in ice-cold RIPA buffer (150 mmol/l NaCl, 50 mmol/l Tris–HCl, 5 mmol/l EDTA, 1% (v/v) Nonidet P-40, 0.5% (w/v) deoxycholate, 10 mmol/l NaF, 10 mmol/l sodium pyrophosphate, 100 mmol/l phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, and 2 μg/ml leupeptin). Proteins were determined by Bradford assay. Aorta extracts (10 μg protein per lane) were mixed with sample loading buffer (B7703, BioLabs) and separated on 12% SDS-polyacrylamide gel. Proteins were electrotransferred onto PVDF membrane (Immun-Blot® 0.2 μm, Bio-Rad). The bands were detected using chemiluminescence assay (ECL + Plus, Amersham). We used a mouse monoclonal antibody for detection of eNOS protein expression (N-30020, Transduction Laboratories) and a polyclonal rabbit antibody against eNOS phosphorylated at Ser^{1177} (9571, Cell Signaling Technology).

2.8. Substances

Unless otherwise stated, all chemicals were obtained from Sigma (Deisenhofen, Germany) in the highest purity available.

2.9. Statistics

Values are means ± S.E.M. for curves and bar graphs. Relaxant responses were given as percentage relaxation relative to the preconstriction level. Statistical analysis was performed by repeated measures ANOVA followed by Tukey–Kramer multiple comparisons test. O_2^- formation was analyzed by ANOVA followed by a Tukey post hoc test where appropriate; $p < 0.05$ was considered statistically significant.

3. Results

Blood glucose levels and body weight, as well as platelet and leukocyte counts, are shown in Table 1. Diabetes-induced increases in blood glucose and reduction in body weight were unaffected by rosuvastatin treatment. Platelet and leukocyte numbers were similar in all groups.

Table 1 – Global parameters in control and diabetic (STZ) rats treated either with placebo or rosuvastatin (RSV)

	Control placebo	STZ placebo	STZ RSV
N	10	12	10
Blood glucose (mmol/l)	9.6 ± 0.9	27.6 ± 0.6*	26.3 ± 1.3*
Body weight (g)	354 ± 6	243 ± 4*	255 ± 10*
Platelets (×1000/μl)	660 ± 40	545 ± 47	599 ± 60
Leukocytes (×1000/μl)	5.7 ± 0.3	4.9 ± 0.5	4.7 ± 0.3

* $p < 0.01$ vs. control placebo.

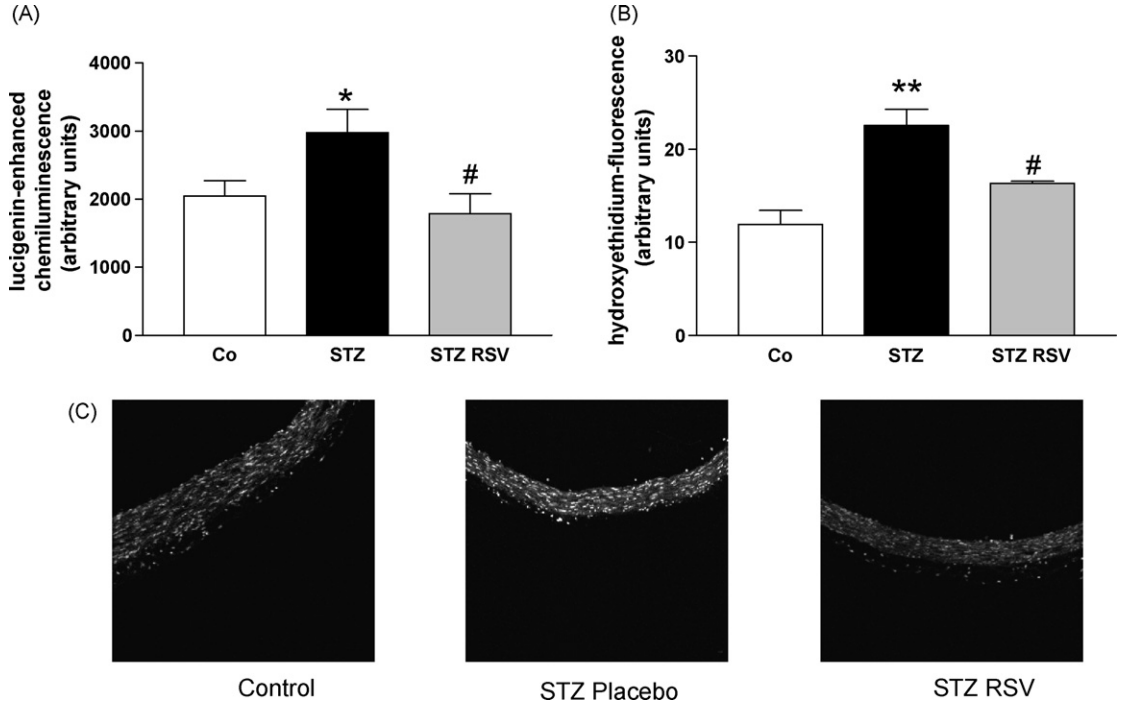

Fig. 1 – O_2^- production in aortic rings from control rats and diabetic rats (STZ) treated either with placebo or rosuvastatin (RSV) was detected and quantified by lucigenin-enhanced chemiluminescence (A) as well as hydroethidium oxidation (B). Mean ± S.E.M. from 6 to 10 separate experiments. Confocal microscopy of 10-μm-thick aortic sections incubated with the fluorescent dye hydroethidium to visualize O_2^- formation throughout the vascular wall (C). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control; $^{\#}p < 0.05$ vs. STZ-placebo.

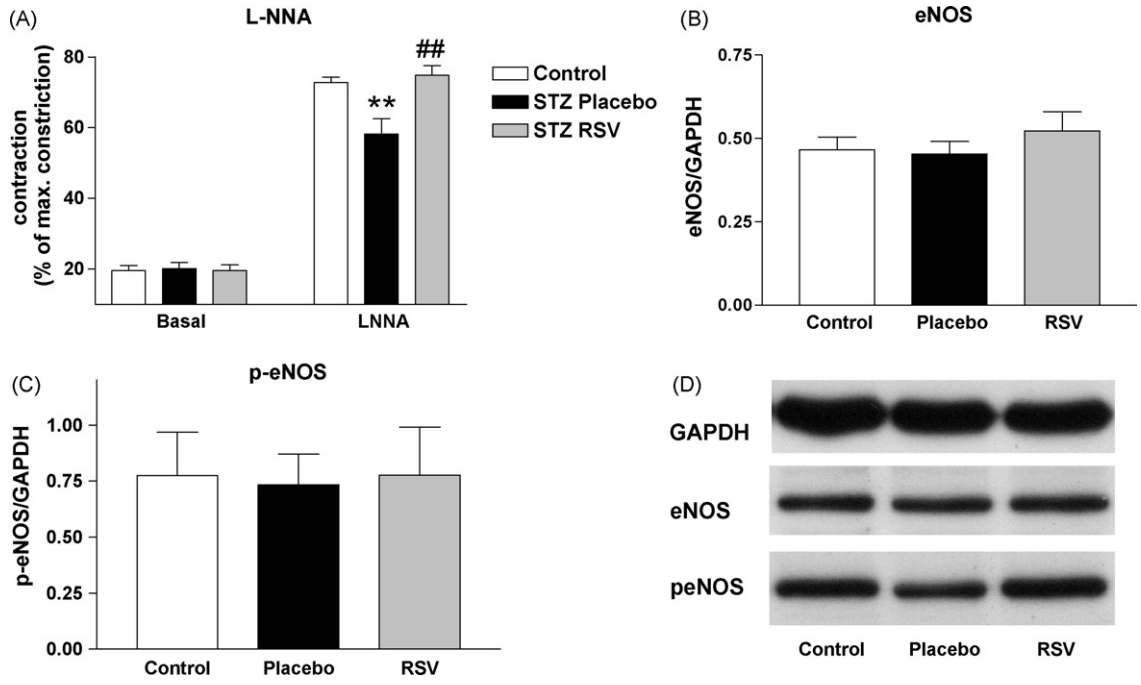

Fig. 2 – Additional increments in vasomotor tone in slightly preconstricted aortic rings (approximately 20% of maximal constriction) following NOS-inhibition with L-NNA were used as an index of vascular contraction-induced calcium-independent NO formation (A). Data are means ± S.E.M. from 10 to 16 different animals, $^{**}p < 0.01$ vs. control; $^{\#\#}p < 0.01$ vs. STZ-placebo. eNOS protein expression (B) and eNOS phosphorylation at Ser1177 (C) were not altered between the three treatment groups, $p > 0.05$, $n = 6$. Representative western blots are shown for eNOS, phospho-eNOS, and GAPDH (D).

3.1. Vascular reactive oxygen species

Aortic O_2^- production, assessed by lucigenin-enhanced chemiluminescence, was significantly increased in rats with diabetes and normalized by treatment with rosuvastatin (Fig. 1A). Hydroxyethidium fluorescence was similarly increased in diabetic aortic rings and reduced by rosuvastatin (Fig. 1B). Representative microtopographic images of O_2^- formation in vascular rings demonstrated increased signal intensity in diabetic versus control animals, which was markedly reduced in rats treated with rosuvastatin (Fig. 1C).

3.2. eNOS and NOS inhibitor-induced vasoconstriction

We assessed contraction-induced NO formation by inhibition of tonic NO release using L-NNA in slightly preconstricted aortic rings as previously described [23]. This caused an additional contraction, which was attenuated in animals with diabetes indicating a reduction of calcium-independent NO release in diabetic aortae. In rosuvastatin-treated diabetic animals, L-NNA-induced constriction was increased to levels comparable with control rats (Fig. 2A). eNOS expression (Fig. 2B and D) as well as eNOS phosphorylation at serine 1177 (Fig. 2C and D) were not altered between the treatment groups.

3.3. Vasomotor function–relaxant responses

Administration of acetylcholine in cumulative concentrations for calcium-dependent activation of eNOS induced an endothelium-dependent vasorelaxation, which was impaired in diabetes and significantly improved by treatment with rosuvastatin (Fig. 3A and C).

The concentration response curve for the NO-donor DEA-NONOate, which was used to assess endothelium-independent vasorelaxation, was shifted to the right in aortae from diabetic rats. Endothelium-independent relaxation was normalized (leftward shift) following treatment with rosuvastatin (Fig. 3B and D).

3.4. Platelet activation

The extent of *in vivo* platelet activation was measured by analysis of platelet-bound fibrinogen reflecting glycoprotein IIb/IIIa activation (Fig. 4A and B) and surface expression of P-selectin as a marker of platelet degranulation (CD62P, Fig. 4C) in unstimulated whole blood. Platelet-bound fibrinogen and surface-expressed P-selectin were both significantly increased in placebo-treated diabetic animals and reduced by chronic treatment with rosuvastatin. The amount of circulating platelet-derived microparticles in whole blood, determined

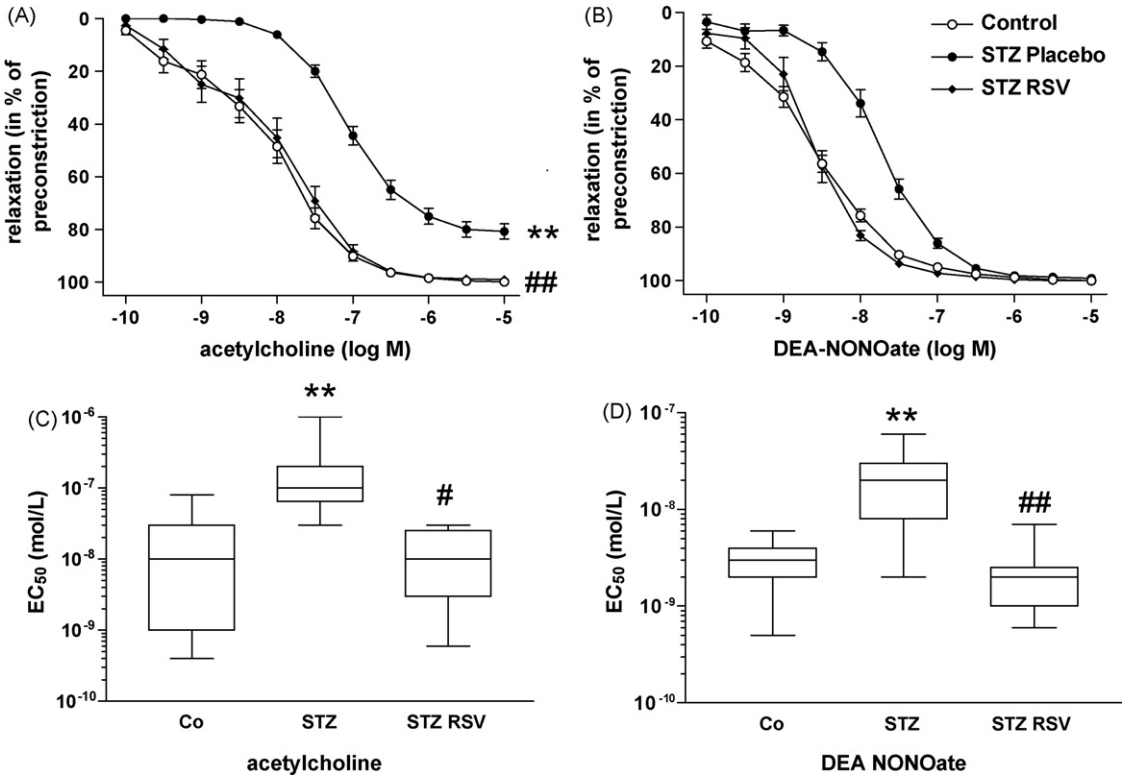

Fig. 3 – Concentration-response curves for endothelium-dependent, calcium-dependent vasorelaxation elicited by cumulative application of acetylcholine (A) and endothelium-independent relaxation by incremental concentrations of DEA-NONOate (B) in isolated aortic rings from control rats and diabetic rats (STZ) treated either with placebo or rosuvastatin (RSV). Respective EC_{50} values were determined for every single concentration response of acetylcholine (C) and DEA-NONOate (D). Data are means ± S.E.M. from 10 to 16 different animals, $^{**}p < 0.01$ vs. control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. STZ-placebo.

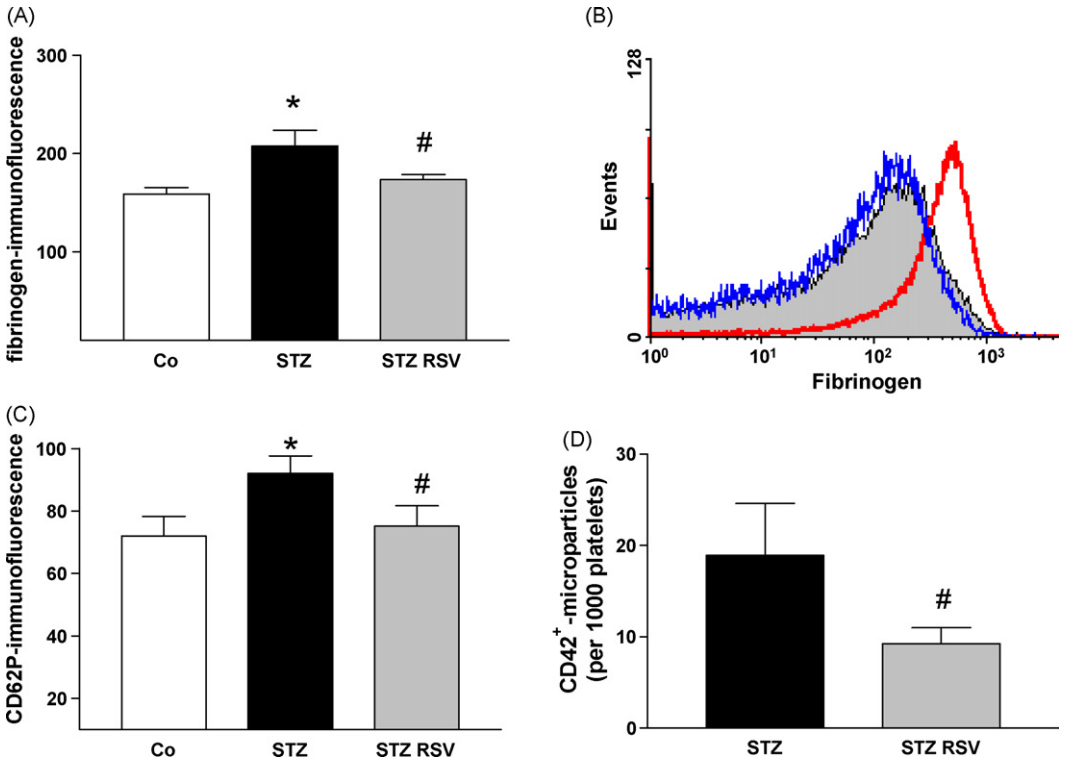

Fig. 4 – Platelet activation was determined by fibrinogen-binding on activated glycoprotein IIb/IIIa (A and B) and surface-expression of P-selectin (CD62P, C) on platelets from control rats or diabetic rats (STZ) treated either with placebo or rosuvastatin (RSV). Typical flow cytometry histograms for platelet-bound fibrinogen (B) show the rightward shift in diabetes (red curve) and a leftward shift following treatment with RSV (blue line) compared to placebo (filled grey). The amount of circulating platelet-derived microparticles in whole blood was determined as the $CD42^+$ fraction of circulating microparticles (D). Results are expressed as the mean fluorescence ± S.E.M. from 10 to 16 separate animals. $^*p < 0.05$ vs. control; $^\#p < 0.05$ vs. STZ-placebo.

as the $CD42^+$ fraction of circulating microparticles, was significantly reduced by rosuvastatin (Fig. 4D).

4. Discussion

In this study we demonstrate that HMG-CoA-reductase inhibition in experimental diabetes improves endothelial function and reduces platelet activation, suggesting that enhanced NO bioavailability contributes to statin effects on platelets in diabetes.

Endothelial dysfunction is a common feature in cardiovascular diseases characterized by an imbalance between NO and ROS. Oxidant stress is a major cause of reduced endothelial NO bioavailability in diabetes [24]. We found significantly higher formation of O_2^- in aortic segments from diabetic rats compared to healthy controls. In parallel, smooth muscle reactivity to exogenous NO was significantly attenuated, contributing to reduced effects of endothelium-derived NO. Both features were successfully reversed by chronic treatment with rosuvastatin. While short-term treatment with a statin reduced oxidative stress in STZ-induced diabetes [25], this study provides evidence for chronic pleiotropic but not direct anti-oxidant effects of rosuvastatin [26,27], which lead to reduced oxidative stress in experimental diabetes.

Several studies have indicated a potential role of eNOS-derived superoxide formation in models of diabetes—the so-called eNOS uncoupling [28–30]. The data in the present study can not specifically rule out eNOS-derived superoxide generation, however, the homogenous transmural signal in the hydroxyethidium images without a pronounced luminal signal (Fig. 1C) suggest that the major source of O_2^- is not the endothelium. In addition, the substantial increase in vasoconstriction by L-NNA in preconstricted aortic rings (Fig. 2) argues against a significant eNOS uncoupling in the present study. Taken together, these results suggest that NO is still produced by eNOS but rather inactivated or scavenged before relaxing smooth muscle cells. Indeed, this is supported by the pronounced rightward shift in the dose response to exogenous NO indicating reduced smooth muscle-sensitivity to NO (Fig. 3B and D), which by itself contributes to the phenomenon of reduced vasorelaxation by an endothelium-dependent agonist (Fig. 3A and C). In the present study, we found no significant modification of eNOS expression or activation neither following induction of diabetes nor by chronic rosuvastatin treatment (Fig. 2B–D). Upregulation of eNOS has been postulated as a central mechanism of statin action in endothelial cells [31,32]. However, results from such in vitro experiments might not be completely transferable to the in vivo environment, because an active metabolite of

atorvastatin has been reported to have direct anti-oxidant effects, whereas this property is not shared by other statins such as rosuvastatin [26]. Another recent study demonstrated preserved eNOS expression during intestinal ischemia/reperfusion, however, this effect was only observed under pathophysiological conditions of reduced eNOS expression and rosuvastatin treatment of sham-operated rats did not further increase eNOS expression above the levels seen in untreated controls [27]. Another important regulator of eNOS function is its phosphorylation by the protein kinase AKT [33], which was positively modulated by fluvastatin in obese Zucker rats [34]. However, in that study eNOS phosphorylation and eNOS expression were both significantly attenuated in their disease model. Therefore, the fact that we did neither observe reduced eNOS expression nor phosphorylation in the first place might have contributed to the unmodulated eNOS protein expression and phosphorylation detected in our rosuvastatin-treated diabetic rats.

We have previously demonstrated that prevention of endothelial dysfunction in a mouse model of diabetes prevents activation of circulating platelets [17] and direct stimulation of the NO target guanylyl cyclase reverses platelet activation in diabetic rats [35]. Endothelial dysfunction is a very early feature of atherosclerosis, and patients with diabetes have an increased risk of thrombosis and accelerated atherogenesis. Atherosclerosis often precedes the clinical manifestation of diabetes and is most pronounced in patients with undiagnosed diabetes [4,36]. Progression of pro-atherosclerotic vessel wall modification in patients with diabetes is associated with platelet degranulation [37]. Increasing levels of glucose have been identified as independent predictors of platelet-dependent thrombosis in patients with coronary artery disease [38]. Furthermore, markers of platelet activation were already significantly increased in individuals positive for islet cell antibodies before onset of overt diabetes mellitus, indicating that platelet activation occurs very early during the development of diabetes [39]. This is clinically reflected by the fact that patients with type 2 diabetes without prior cardiovascular events have a risk of myocardial infarction similar to that among non-diabetic patients with prior myocardial infarction [2]. Thus, activated platelets have a major impact on morbidity and mortality as most diabetic patients die from cardiovascular atherothrombotic events [40]. We previously demonstrated that prevention of endothelial dysfunction in experimental diabetes protected against enhanced platelet activation *in vivo* due to improved NO bioavailability [17]. In addition to normalization of vascular function, chronic treatment with rosuvastatin-reduced platelet activation *in vivo* as assessed by platelet-bound fibrinogen on activated glycoprotein IIb/IIIa, as well as surface-expressed P-selectin on circulating platelets in diabetic rats in the present study.

Multiple effects of statin treatment have been described in patients with, and in experimental models of hypercholesterolaemia, including reversal of hypercholesterolaemia-associated platelet activation, and reduction of platelet reactivity, thromboxane biosynthesis, thrombin generation, aggregation and thrombogenic potential (as reviewed by [41,42]) as well as platelet thrombus formation [43]. Recently, the CARDS trial demonstrated that statin treatment in normocholesterolaemic patients with type II diabetes mellitus without previous history of cardiovascular disease can lead to a substantial reduction in major cardiovascular events such as acute coronary events and stroke [44]. These results underlined the potential role of statins and their pleiotropic effects for primary cardiovascular disease prevention in diabetics. Significant cardiovascular protection could be observed as early as several months after starting statin treatment [45].

The pleiotropic effects of HMG-CoA-reductase inhibitors in general include improvement of endothelial function, platelet function, and atherosclerotic plaque stability, and suppression of vascular inflammation (as recently reviewed [42,46]). Statins exert several protective effects on the endothelium including reduced activity of NAD(P)H oxidases [47] and increased endothelial NO bioavailability [48] and enhanced endothelial function in healthy animals, indicating cholesterol-independent effects on NO bioavailability [47]. Increased lipid levels are commonly observed in patients with type 2 diabetes as part of the metabolic syndrome [49,50] and statin treatment is recommended under these conditions [19,51]. In addition, statin treatment is discussed as a method of primary prevention therapy in normolipidaemic type II diabetics [44]. In the present study, we demonstrate that chronic treatment with rosuvastatin beneficially modulates platelet activation and vascular dysfunction in experimental diabetes.

5. Conclusion

Diabetes is associated with endothelial dysfunction and platelet activation, which are positively modulated by chronic treatment with rosuvastatin. These effects may contribute to the reduction of cardiovascular events by statins in diabetic patients.

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