

# Effect of Antihypertensive Treatment with Doxazosin on Insulin Sensitivity and Fibrinolytic Parameters

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## Summary

The effects of the selective alpha-1-adrenoceptor antagonist doxazosin on metabolic and fibrinolytic parameters were studied in hypertensive patients with various degrees of fasting plasma insulin levels (Group A:  $22.5 \pm 3 \mu\text{U/ml}$ , Group B:  $8.1 \pm 1.5 \mu\text{U/ml}$ ;  $p < 0.01$ ) to disclose a potential link between a doxazosin-induced alteration of insulin and/or lipid metabolism and possible changes of these parameters on the fibrinolytic system. Doxazosin treatment resulted in a dose-dependent reduction of basal insulin levels in group A to  $16 \pm 3 \mu\text{U/ml}$ ;  $p < 0.05$ . This finding was paralleled by a dose-dependent increase in t-PA<sub>mass</sub> concentration in the same patient group (basal t-PA<sub>mass</sub> from  $9.7 \pm 1$  to  $15.5 \pm 2 \text{ ng/ml}$ ;  $p < 0.05$ ). As PAI-1 "active" as well as total antigen levels were not altered in parallel, the net effect on the endogenous fibrinolytic system is an increase of the fibrinolytic potential.

## Introduction

A decreased insulin sensitivity with consecutive hyperinsulinemia is suggested to be the possible link between hypertension, obesity, and glucose intolerance, all of them independent risk factors for CAD (1, 2). Furthermore, insulin resistance has been shown to be related to a low fibrinolytic activity (3-6) which in turn is associated with an increased incidence of atherothrombotic disorders (7, 8)

The alpha-1-receptor antagonist doxazosin has been shown to improve the insulin sensitivity and to decrease plasma insulin levels (9-13). Furthermore, doxazosin has been shown to produce positive effects on parameters of the fibrinolytic system (12, 13). It was suggested that these findings were related to the reduction in blood pressure and/or to changes in triglyceride plasma levels (13). Several studies have demonstrated the close relationship between plasma insulin levels and parameters of the endogenous fibrinolytic system (3, 14-16). There exist, however, no clinical investigations about possible relationships between doxazosin-induced changes in glucose and insulin metabolism and changes in the fibrinolytic system. Therefore, we investigated the possibility of such relationships at basal conditions and during intravenous glucose tolerance tests in two matched patient groups with CAD, borderline to mild arterial hypertension with different fasting plasma insulin levels.

## Patients and Methods

*Patient selection and clinical characteristics.* A total of 17 consecutive moderately obese, male patients with borderline to mild hypertension according to the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (17) were found eligible for participating in this study. In all patients CAD was proven angiographically and all patients had stable angina (CCS class I and II). Patients were free of other diseases as determined by case history, physical examination and laboratory investigations of renal, hepatic and thyroid function, respectively. While all patients were moderately obese and hyperlipidemic two groups of patients were defined according to their fasting plasma insulin levels (Group A: fasting plasma insulin  $> 15 \mu\text{U/ml}$ ; group B:  $< 15 \mu\text{U/ml}$ ) (18). This design was chosen to evaluate both, doxazosin induced changes in blood pressure and plasma insulin on concomitant changes in fibrinolytic parameters: while blood pressure reduction was most likely to be observed in both groups, reduction of plasma insulin levels and possible changes of fibrinolytic parameters expectedly only would occur in the group with increased plasma insulin levels.

The investigation conformed with the principles outlined in the Declaration of Helsinki and the study protocol was approved by the local human subjects committee. Written informed consent was obtained from all patients. Patients were asked not to alter their dietary habits and physical behaviors for the duration of the study. Two patients dropped out of the study, one due to drug-related postural dizziness and the other one because of infrequent intake of doxazosin during the study. Therapy did not show any other side effects in the remaining patients.

All patients included into the study were non-smokers. As shown in Table 1, both patient groups were comparable with respect to clinical variables except weight which was significantly higher in group A.

*Study design.* This prospective, open study lasted five months and was divided into four phases (Fig. 1): phase A consisted of a four-week washout period. During this period all antihypertensive or accompanying medication was stopped except aspirin (100 mg/d). Intake of sublingual nitroglycerin was allowed in case of anginal pain. Phase B consisted of 6 weeks during which patients were treated with 2 mg/d doxazosin in one single dose (Supressin®, Pfizer, Austria). In Phase C, which also lasted for 6 weeks, doxazosin dosage was increased to 4 mg/d (single dose). Phase D was a second washout period after cessation of doxazosin treatment. Clinical investigations, blood sampling for laboratory evaluations and intravenous glucose tolerance tests (ivGTT) were performed at the end of each study phase.

*Blood sampling, intravenous glucose tolerance test, and blood pressure measurement.* Blood was collected at 8.00 a.m. after a 14 h overnight fast before intake of doxazosin to exclude possible effects of circadian variation of fibrinolytic parameters on the results (19, 20) with the patient in recumbent position. Blood was drawn from the forearm with minimal venous stasis into plastic tubes prepared with EDTA (final concentration  $5 \times 10^{-2} \text{ M}$ ) for determination of fibrinolytic parameters or with citrate (0.11 M final concentration) for all other biochemical measurements and centrifuged immediately ( $3000 \times g$ ,  $4^\circ \text{ C}$ , 10 min). Supernatants were discarded and stored in 0.5 ml aliquots at  $-70^\circ \text{ C}$  until use. Following basal blood collection, ivGTT was performed as

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Table 1 Clinical characteristics of the two patient groups

Characteristics	Group A	Group B	p-value
patient number	7	8	
age (years)	55±3	60±3	n.s.
weight (kg)	95.2±5.0	84.7±2.4	0.05
height (cm)	178±4	174±2	n.s.
body mass index (BMI)	29.7±1.0	28±0.6	n.s.
waist-hip ratio	0.97±0.02	0.96±0.01	n.s.
SBP (mmHg)	139±6	148±8	n.s.
DBP (mmHg)	85±2	86±3	n.s.

Values are given as mean±SD. SBP denotes systolic blood pressure, DBP denotes diastolic blood pressure. n.s. denotes not significant.

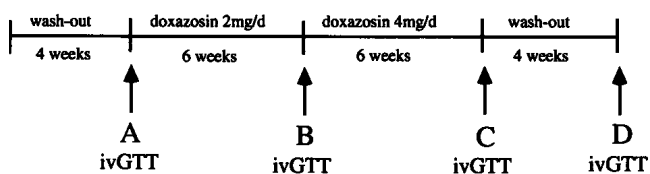


Fig. 1 Study design. A, B, C, and D denote time points of blood sampling at the end of each study phase. Phase A = first washout period; Phase B = 6 weeks of doxazosin therapy using 2 mg/day; Phase C = 6 weeks of doxazosin therapy using 4 mg/day; Phase D = second washout period after cessation of doxazosin treatment

described (21): after injection of 300 mg glucose/kg (33% glucose solution) within 1.5 min, blood samples were drawn after 5, 10, 30, 60, 90, and 120 min, respectively. Blood pressure was measured three times in recumbent position after a rest of 10 min and the mean of the 3 measurements was used.

**Determination of biochemical parameters.** Plasma levels of triglycerides (22) total cholesterol (23) as well as HDL and LDL cholesterol (24) were determined in all blood samples taken at rest by use of standard techniques as indicated. Furthermore, glucose (25) and insulin (26) levels as well as tissue-type plasminogen activator mass concentration (t-PA<sub>mass</sub>) (27) and type-1 plasminogen activator inhibitor (PAI-1) "active" antigen (by use of an ELISA system including a monoclonal antibody directed against the active center of the PAI-1 molecule) and PAI-total antigen were determined (28). Insulin concentrations were measured with a commercial radioimmunoassay (Serono Diagnostics®). The intra and interassay coefficient of variation were 4.5% and 4.5% for higher concentrations and 7.4% and 8.0% for lower insulin concentrations. Inter and intraassay coefficient of variation for fibrinolytic parameters (Technoclone®) were 10% and 5%, respectively for all assays used.

**Statistical analysis.** If not indicated otherwise, data are expressed as mean ± SEM. To disclose Gaussian or non-Gaussian distribution the Kolmogorow-Smirnoff test was performed on all variables analyzed. Non-parametric statistical methods were used in all variables which exhibited a non-Gaussian distribution, i.e. t-PA, and PAI-1 plasma levels, respectively. To compare the differences within groups between variables before and after doxazosin treatment analysis of variance (ANOVA) for repeated measures was used for normally distributed variables while non-Gaussian distributed variables were analyzed by means of the Wilcoxon test. The difference of variables between the two groups was compared either by univariate analysis (Student's t-test) or by means of the Mann-Whitney test. Selected variables were correlated using Spearman's rank coefficient. Significance was accepted at the p < 0.05 level for all analyses. All statistical calculations were performed using a computer program (MacIntosh, StatView 4.0, System 7.0.1).

## Results

**Effects of doxazosin on systolic and diastolic blood pressure.** As shown in Fig. 2, doxazosin decreased both systolic and diastolic blood pressure. Blood pressure reduction was significant only after administration of 4 mg/day of doxazosin. After withdrawal of doxazosin a significant re-increase of both systolic and diastolic blood pressure to pre-treatment values could be demonstrated for both groups.

**Effects of doxazosin on lipids.** Table 2 summarizes the results of doxazosin treatment on blood lipids. As can be seen, no significant alterations of any of these variables could be demonstrated between the study groups for the different phases of the study. Triglyceride levels exhibited an inconsistent response to doxazosin in the study groups: a non-significant decrease in group A followed by a re-increase after cessation of therapy as well as a non-significant increase in group B.

**Effects of doxazosin on plasma glucose levels.** Plasma glucose levels at rest were comparable between both groups and did not show signifi-

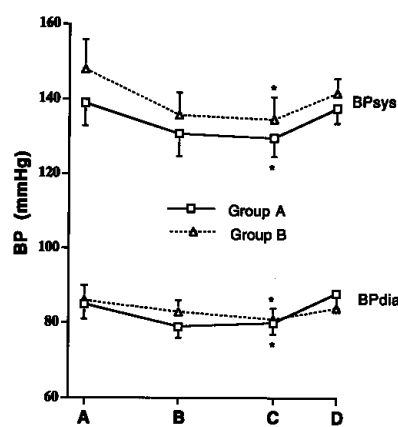


Fig. 2 Systolic and diastolic blood pressures (mean ± SEM) after the different study phases (A-D) for the two patient groups. BP denotes blood pressure, SBP denotes systolic blood pressure, DBP denotes diastolic blood pressure. The asterisk (\*) indicates a p-value of < 0.05

Table 2 Levels of blood lipids after the different study phases (A-D) for the two patient groups

Variables	Phase	Group A	Group B	p
total	A	239±11	242±11	n.s.
cholesterol	B	236±8	246±16	n.s.
(mg/dl)	C	238±11	263±17	n.s.
	D	243±19	220±18	n.s.
HDL-	A	40±1	45±3	n.s.
cholesterol	B	40±3	46±4	n.s.
(mg/dl)	C	37±3	46±5	n.s.
	D	33±3	42±5	n.s.
LDL-	A	157±3	166±10	n.s.
cholesterol	B	164±8	159±18	n.s.
(mg/dl)	C	161±9	193±32	n.s.
	D	163±11	151±15	n.s.
triglycerides	A	222±58	156±17	n.s.
(mg/dl)	B	214±93	191±28	n.s.
	C	178±51	216±29	n.s.
	D	259±72	215±33	n.s.

Values are given as mean±SEM; n.s. denotes not significant.

cant alterations during doxazosin therapy (Group A: A:  $96 \pm 7$  mg/dl; C:  $96 \pm 6$  mg/dl; Group B: A:  $103 \pm 7$  mg/dl, C:  $105 \pm 8$  mg/dl). During ivGTT glucose levels increased with a similar extent in both study groups, showing peak levels after 5 min and a comparable decrease over time thereafter. Regardless of the dosage used, doxazosin had no significant effect on glucose plasma levels during ivGTTs in both groups (data not shown).

**Effects of doxazosin on plasma insulin levels.** Fasting insulin levels were significantly higher in group A:  $22.5 \pm 3$   $\mu$ U/ml vs. group B:  $8.1 \pm 1.5$   $\mu$ U/ml;  $p < 0.01$ . While doxazosin 2 mg/d showed no effects on plasma insulin in both groups, in group A doxazosin treatment with 4 mg/d resulted in a significant decrease in insulin levels to  $16 \pm 3$   $\mu$ U/ml;  $p < 0.01$  (Fig. 3). The area under the insulin concentration time curve decreased significantly only during doxazosin treatment with 4 mg/d from  $6946 \pm 890$  to  $4214 \pm 559$  ( $p < 0.05$ ) in group A, while it remained unchanged in group B (from  $2334 \pm 428$  to  $2778 \pm 244$ ; n.s.).

**Effects of doxazosin on t-PA<sub>mass</sub>.** As can be seen from Fig. 3 fasting levels of t-PA<sub>mass</sub> were similar in both groups. In group A after phase C doxazosin (4 mg/d) exhibited a significant increase in basal t-PA<sub>mass</sub> from  $9.7 \pm 1$  to  $15.5 \pm 2$  ng/ml. This effect could not be obtained when doxazosin was used in a dosage of 2 mg/d (Phase B) and was completely reversible after withdrawal of the drug (Phase D). In both groups t-PA<sub>mass</sub> continuously decreased during ivGTT (data not shown).

**Effects of doxazosin on PAI-1 total and PAI-1 "active" antigen levels.** At the end of the different study phases (A-D), both PAI-1 total and "active" antigen levels exhibited no significant differences neither at rest (Fig. 3) nor at the different time points of blood collection during ivGTT's in both study groups (data not shown). The results of PAI-1 active antigen paralleled the data of PAI-1 total antigen but at an around 3-5-fold lower niveau. Plasma levels of PAI-1 total as well as "active" antigen decreased constantly over time during ivGTT's after all phases and in both groups (data not shown).

## Discussion

The present study demonstrates the metabolic and fibrinolytic effects of the selective alpha-1-adrenoceptor antagonist doxazosin in patients with a cluster of concomitant cardiovascular risk factors. One of the principal findings of this study was that doxazosin treatment resulted in a reduction of insulin levels in patients with increased basal plasma insulin levels with a parallel increase of t-PA<sub>mass</sub>, an effect which was reversible after cessation of therapy. As PAI-1 levels were not increased concomitantly, the net effect on the fibrinolytic system is an improvement of its potential.

**Effect of doxazosin treatment on plasma insulin levels.** Our results are in accordance to previous studies demonstrating that doxazosin treatment beneficially affects insulin plasma levels: fasting insulin levels were decreased and early as well as late insulin response during ivGTT was improved. Thereby, a significant reduction of plasma insulin could only be observed in patients with increased fasting insulin plasma levels. The mechanisms underlying the beneficial effect of doxazosin on insulin metabolism are still not clarified: it may be speculated that relaxation of systemic arterioles increases muscle blood flow thus improving tissue response to insulin and glucose (29, 30). This hypothesis is in accordance with our finding that a significant improvement of insulin metabolism could only be detected in patients with increased fasting plasma insulin levels treated with the higher dose of doxazosin (4 mg/d) which also caused a significant drop of blood pressure. A direct biochemical effect of doxazosin on insulin is unlikely because neither dose of doxazosin was effective in group B.

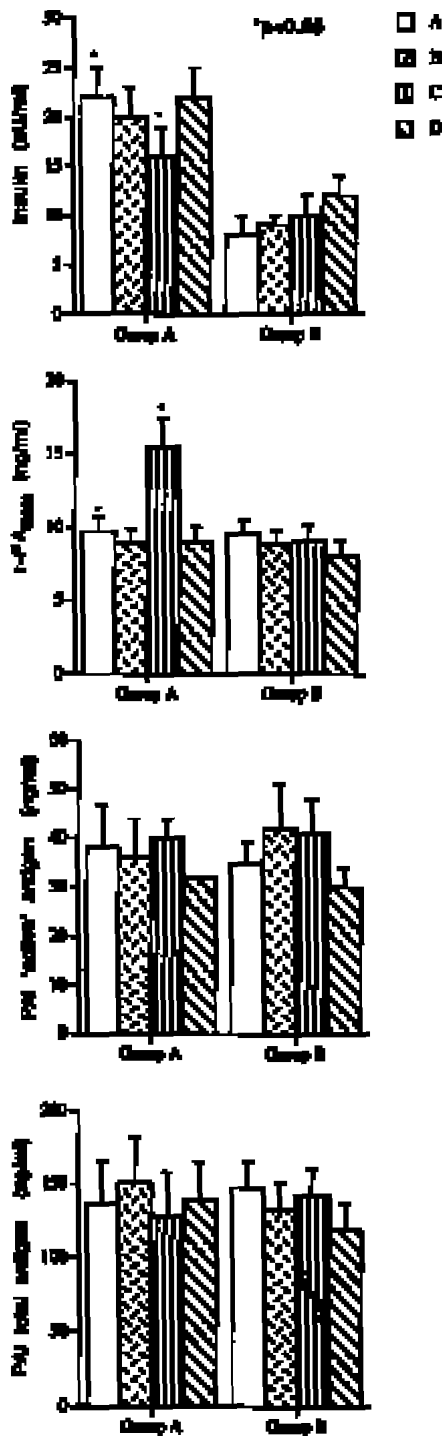


Fig. 3 Fasting plasma levels of insulin, t-PA<sub>mass</sub>, PAI-1 "active" antigen and PAI-1 total antigen after the different study phases (A-D) for both patient groups. Values represent mean  $\pm$  SEM. The asterisk (\*) indicates a p-value of  $< 0.05$  for the comparable time points of blood collection during ivGTT between the different phases (A-D) of the study

**Effect of doxazosin treatment on parameters of the fibrinolytic system.** An impaired endogenous fibrinolytic potential as mainly determined by low t-PA and high PAI-1 plasma levels (31) bears a higher risk for the development and the clinical course of coronary artery disease by inducing a prothrombotic state. Indeed, numerous studies have addressed this question and clearly demonstrated that PAI-1 elevations (4, 32-34) as well as parallel elevations of PAI-1 and

t-PA<sub>mass</sub> (35-38) are risk factors for CAD. The concomitant increase of t-PA<sub>mass</sub> and PAI-1 plasma levels can be explained by assay systems used for determination of t-PA<sub>mass</sub>: these assays detect not only free t-PA antigen (= t-PA<sub>mass</sub>) but also t-PA/PAI-1 complexes which strongly depend on the amount of PAI-1 present in the circulation. Measurements of t-PA<sub>mass</sub> as well as PAI-1 antigen together with PAI-1 active antigen, reflecting non-complexed active PAI-1, should allow evaluation of the overall fibrinolytic capacity: Whenever active PAI-1 would increase and concomitantly the relation between t-PA<sub>mass</sub> and total PA-1 antigen would remain constant, a decrease in total fibrinolytic capacity can be expected; in contrast, either a decrease in active PAI-1 antigen or a shift of the relation between t-PA<sub>mass</sub> and total PAI-1 antigen towards t-PA<sub>mass</sub> would reflect an increase in the fibrinolytic potential.

In the present study treatment with 4 mg/d doxazosin resulted in a significant increase of t-PA<sub>mass</sub> concentration without any change in active PAI-1 or total PA-1 antigen in patients with increased basal insulin levels only, therefore indicating an increase in the overall fibrinolytic potential. This effect was reversible after cessation of therapy, suggesting a causal effect of doxazosin; such a causative effect is also suggested by the dose dependency of the changes seen. Furthermore, t-PA<sub>mass</sub> increase was paralleled by a decrease of plasma insulin levels thus suggesting a reverse relation of insulin plasma levels and t-PA<sub>mass</sub>. Comparable data from the literature are rare: in a study including 1500 patients with angina pectoris, Juhan-Vague et al. (39) found a significant positive correlation between plasma insulin levels and PAI-1 as well as t-PA antigen levels and a negative correlation between insulin levels and t-PA activity. As discussed above, this can be explained by the determination of an increased amount of t-PA/PAI-1 complexes by the t-PA assay used. Jansson et al. showed that doxazosin treatment did not influence basal fibrinolytic parameters in moderately hypertensive and hyperlipidemic patients but the response of t-PA activity to venous occlusion was improved after 6 months of treatment (13). The authors suggested that this effect was related to the parallel reduction of triglyceride levels. However, plasma insulin levels were not investigated in that study.

It is unlikely that changes in t-PA<sub>mass</sub> in group A was influenced mainly by blood pressure reduction which occurred in both groups or by alterations of triglyceride levels which remained unchanged during doxazosin treatment. Furthermore, a direct drug effect of doxazosin on t-PA<sub>mass</sub> would have led to changes in both study groups which was not the case. Therefore, the doxazosin-induced increase of t-PA<sub>mass</sub> in these patients seems to be mediated via changes of insulin levels. It might be speculated that similar to hyperlipidemia, also chronic elevation of plasma insulin is capable of inducing endothelial dysfunction, resulting in a reversible suppression of endothelial t-PA production and t-PA release thus supporting the development of a prothrombotic/antifibrinolytic state. Accordingly, a reduction of elevated plasma insulin, as shown in this study, and a normalization of increased plasma lipids, as demonstrated recently (40) is potentially able to improve endothelial function and normalize endothelial t-PA production and release.

**Conclusion.** Our data confirm the beneficial effect of the selective alpha-1-blocker doxazosin on plasma levels of insulin in patients with increased fasting plasma insulin levels. This was paralleled by a dose-dependent increase in t-PA<sub>mass</sub> concentration in the same patient group suggesting that doxazosin might also have favourable effects on the endogenous fibrinolytic system.

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