Impact of a combined treatment of angiotensin II type 1 receptor blockade and 3-hydroxy-3-methyl-glutaryl-CoA-reductase inhibition on secretory phospholipase A2-type IIA and low density lipoprotein oxidation in patients with coronary artery disease

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Aims
To evaluate the impact of a combined treatment of angiotensin II type 1 (AT1)-receptor blockade and 3-hydroxy-3-methyl-glutaryl-CoA-reductase inhibition (statin) on the secretory phospholipase A2 type IIA (sPLA2-IIA) and oxidized low density lipoprotein (oxLDL) in patients with coronary artery disease (CAD).

Methods and results
Sixty patients with angiographically documented CAD and a history of arterial hypertension were randomized in a double-blinded fashion to pravastatin (PRAV, 40 mg/day, n = 30) or PRAV plus irbesartan (PRAV + IRB, 40 mg/day + 300 mg/day, n = 30) and were treated for 3 months. Blood pressure (BP) and cholesterol fractions were determined at baseline and after 3 months. SPLA2 activity as primary endpoint, sPLA2-IIA protein, oxLDL levels, and high-sensitivity (hs)-C-reactive protein were measured by an enzyme-linked immunabsorbent assay. In both treatment groups, systolic BP levels and circulating HDL and LDL levels were reduced to the same extent. The combined treatment of PRAV + IRB significantly decreased sPLA2-IIA activity and sPLA2-IIA-protein concentration compared with PRAV treatment alone (P, 0.05). In addition, PRAV + IRB significantly reduced oxLDL levels compared with PRAV treatment alone (P < 0.05). This effect was independent of changes in LDL cholesterol levels.

Conclusion
These findings are consistent with the notion that the combined treatment of pravastatin with irbesartan reduced sPLA2-IIA-activity, sPLA2-IIA-protein concentration, and oxLDL in patients with CAD suggesting a novel anti-atherogenic effect by combining AT1-receptor blockade with statin treatment.

Keywords
AT1-receptor blockade • Statin • oxLDL • sPLA2-IIA • CAD

Introduction
Atherosclerosis with its associated cardiovascular events—myocardial infarction, sudden cardiac death, or stroke—is one of the leading causes of death in the western countries.1 Both, chronic activation of the renin–angiotensin system (RAS) and lipoproteins, especially oxidatively modified low density lipoproteins (oxLDLs) are well-established contributors to the development and progression of atherosclerosis. Interestingly, RAS activation...
as a characteristic feature of the hypertensive cardiovascular system and enhanced LDL levels are often co-existent and may worsen the prognosis of the patients affected.\textsuperscript{2–4} Besides a sole co-existence, direct interactions between a chronically activated RAS and lipoproteins have been indicated, which may further promote atherogenesis. This concurrence may be procured via angiotensin (Ang) II, the effector peptide of the RAS and activation of the angiotensin II type 1 (AT\textsubscript{1})-receptor.\textsuperscript{5} Thus, it is not surprising, that some experimental as well as clinical evidence support the notion that the combination of 3-hydroxy-3-methyl-glutaryl (HMG)-CoA-reductase inhibition (statin) with an AT\textsubscript{1}-receptor antagonist may act additively.\textsuperscript{6–9,10} However, the underlying mechanisms as well as the clinical impact of such an adjunction remain a matter of ongoing debate. In this regard, recent findings by our group indicated that Ang II-mediated LDL oxidation seemingly depends on the expression and activity of the secretory phospholipase type IIa (sPLA\textsubscript{2-IIa}), an acute-phase reactant and independent predictor for coronary events in healthy subjects and patients with documented coronary artery disease (CAD).\textsuperscript{11–13} sPLA\textsubscript{2-IIa} hydrolyses cell membrane phospholipids, leading to the formation of free fatty acids and lysophospholipids which are precursors of pro-inflammatory prostaglandins, leukotrienes, and platelet-activation factor. These factors enhance lipid aggregation and LDL oxidation and stimulate oxLDL uptake by macrophages, important aspects of atherosclerotic plaque development.\textsuperscript{14–16} In the circulation, sPLA\textsubscript{2} directly hydrolyses LDL, which leads to the formation of oxidation-susceptible, small-dense LDL particles with altered configuration of apolipoprotein B, resulting in an LDL-receptor-independent cellular uptake of lipoproteins. Interestingly, histological studies of human atherosclerotic plaques revealed a positive correlation between sPLA\textsubscript{2-IIa} expression and disease severity.\textsuperscript{17} Furthermore, sPLA\textsubscript{2-IIa} was associated with an increased risk for restenosis after percutaneous transluminal coronary angioplasty.\textsuperscript{18} On the basis of this evidence, we hypothesized that sPLA\textsubscript{2-IIa}—by mediating Ang II-dependent LDL oxidation—might be involved in the interactions between RAS activation and hypercholesterolaemia and therefore represent an attractive new target in the treatment of patients with CAD. Thus, we investigated whether an AT\textsubscript{1}-receptor blockade on top of a standard statin treatment elicits additional effects on circulating sPLA\textsubscript{2-IIa} and oxLDL in patients with established CAD.

**Methods**

**Patients**

All men and women aged between 35 and 75 consecutively admitted for elective percutaneous coronary intervention (PCI) to the Department of Cardiology and Angiology of the Hannover Medical School (MHH), Germany, with CAD and a history of arterial hypertension were candidates for inclusion into the study. The screening period of time was between April and October 2005. Patients with chronic renal failure, LDL serum levels $>$155 mg/dL, or hypotension [systolic blood pressure (BP) $<$90 mmHg] as well as with insulin-dependent diabetes, chronic inflammatory, or malignant diseases were excluded. Patients already receiving statins, AT\textsubscript{1}-receptor antagonists or ACE-inhibitors, non-steroidal anti-inflammatory drugs (other than 100 mg of acetylsalicylic acid), corticosteroids, cytostatic agents, or patients who received a drug-eluting stent were also excluded. The patients were asked to give their written informed consent to participate after PCI. Among 237 patients listed for PCI, finally 62 patients left to be enrolled in the present study 6 weeks after PCI following a computer-generated randomization list. All patients had angiographically documented CAD without residual flow-limiting coronary stenosis (no coronary stenosis $>$50%) after the performed PCI, an ejection fraction $\geq$55% and normal exercise stress test prior to randomization as required safety parameter. None of the patients reported symptoms of angina pectoris or heart failure at the time of inclusion into the study. The patients’ screening and inclusion procedure as well as patients’ follow-up is shown in Figure 1.

The study was approved by the local Ethics Committee (#3059/2003) and all patients gave their written informed consent to participate.

**Study objectives and study protocol**

The current study was aimed to investigate whether an AT\textsubscript{1}-receptor blockade on top of a standard statin treatment elicits additional effects on circulating sPLA\textsubscript{2-IIa} and subsequently on lipid-peroxidation products such as oxLDL and established markers of inflammation such as high-sensitivity (hs)-C-reactive protein in patients with established CAD.

We defined one primary endpoint (baseline adjusted treatment effect of between-group comparisons for lowering sPLA\textsubscript{2-IIa} activity after 3 months) and six secondary endpoints (treatment effects for lowering blood pressure and sPLA\textsubscript{2}-protein, oxLDL, LDL HDL, and hs-C-reactive protein concentrations).

Patients were randomly assigned in a 1:1 ratio to receive either pravastatin ($n=32$ patients, 40 mg/day, group A, PRAV) or pravastatin plus irbesartan ($n=30$, 40 mg/day pravastatin and 300 mg/day irbesartan, group B, IRB+i-PRAV) in a double-blinded fashion following sequentially a randomization list without using blocks or stratifications. The study drug was blinded by the hospital pharmacy. All study personnel and patients were blinded to treatment assignment. There were identical sequentially numbered boxes (after unblinding = group A, pravastatin/placebo, and = group B, pravastatin+irbesartan); each box containing two bottles labelled as ‘medication A’ (after unblinding = pravastatin) and ‘medication B’ (after unblinding = either irbesartan or placebo). ‘Medication B’ was uptitrated after 2 weeks. Accordingly, patients received irbesartan at the starting dose of 150 mg and were uptitrated to 300 mg after 2 weeks. Pravastatin was administered at the dosage of 40 mg/day during the entire study. Both treatments were administered for 3 months. The groups were unblinded at the end of the entire study. (Figure 2). As shown in Figure 1, two patients (after unblinding each from the PRAV group) dropped out 2 and 9 days after starting study medication without giving a reason. We did not include these two patients in our analysis because this might potentially alter baseline levels but parameters at 3 months would not change with regard to the final outcome based on the fact that they factually did not have really started the study.

**Blood sampling and laboratory analysis**

Serum samples were collected at baseline and after 3 months. Blood samples were drawn in a seated position from the antecubital vein and serum samples were stored at $-80^\circ$C before use. Serum sPLA\textsubscript{2-IIa} protein and sPLA\textsubscript{2} activity, hs-C-reactive protein and oxLDL were measured at baseline and at 3 months of follow-up using an enzyme-linked immunosorbent assay (ELISA) technique. Non-invasive blood pressure measurement was performed in seated position by Riva-Rocci/Korotkow’s method.
Study personnel and patients were blinded to the results of the laboratory analysis performed at the end of the entire study. Only the study data monitoring personnel saw unblinded data with regard to safety parameters but none had any contact with the patients.

**High-sensitivity-C-reactive protein analysis**
Plasma samples obtained at baseline and at 3 months were thawed and assayed for hs-C-reactive protein using a high-sensitivity enzyme immunoassay with a coefficient of variation below 5% (Dade Behring using a BN II nephelometer analyzer, FDA-approved). The limits of detection (0.02 mg/L) and quantification (0.15 mg/L) were reported recently.12 Every experiment was performed in triplicates. Data are given as mean ± SEM.

**Secretory phospholipase A2 type-IIA assays**
Plasma samples obtained at baseline and at 3 months were thawed. SPLA2-IIA ELISA (Cayman Chemical Company, Ann Arbor, MI, USA) was performed to determine the protein concentration in serum samples and sPLA2 activity was assessed in serum samples by a commercially available kit (Quantikine R&D Systems, Minneapolis, Minnesota) following the recommendations of the manufacturer. Every experiment was performed in triplicates (Fluostar Galaxy, BMG Lab technologies) and the results are given as mean ± SEM. Intra-assay variability from triplicate analysis was below 1% in all samples.

**Determination of oxidized low density lipoprotein**
OxLDL were determined in EDTA-plasma of patients by ELISA technique (Mercodia) utilizing a specific murine monoclonal antibody mAb-4E6.19 OxLDL ELISA was performed following manufacturer's recommendations; each measurement was performed in triplicates (Fluostar Galaxy, BMG Lab technologies).

**Statistical analysis**
All statistical analyses were performed by the Department of Biometrics. The primary endpoint was the baseline-adjusted treatment effect of between-group comparisons for lowering sPLA2-activity after 3 months. Secondary endpoints were defined as baseline-adjusted between-group treatment effects for lowering blood pressure and sPLA2 protein, oxLDL, LDL, HDL, and hs-C-reactive protein levels. Data were analysed by ANCOVA using the SAS procedure MIXED.
All tests were performed two-sided and \( P < 0.05 \) were considered as statistically significant.

Sample size was calculated for an independent t-test of the means post-therapy with \( \alpha = 0.05 \). With a sample size of 27 per group, a relative effect of 80% of the standard deviation is detectable with a power of 80%. Thus, with an \( \alpha \) priori estimated standard deviation of 35 U/mL, a difference of about 28 U/mL is detectable for the primary endpoint sPLA2 activity which we consider as clinical relevant.

**Results**

**Clinical characteristics**

Both PRAV- and PRAV+IRB-treated groups did not differ with regard to gender, body mass index, cardiovascular risk factor profile, or medication (Table 1). Four patients in the PRAV+IRB group and two patients in the PRAV group received oral anti-diabetic medication for non-insulin-dependent diabetes. No patient received AT\(_1\)-receptor antagonists, ACE-inhibitors, or statins prior to inclusion into the study. Patients with CAD and a history of arterial hypertension received standard treatment with 100 mg of acetyl salicylic acid, as well as anti-hypertensive treatment with beta-blockers, calcium channel antagonists, and diuretics during the 6 weeks from screening to therapy onset as summarized in Table 1. None of the patients reported symptoms of angina pectoris or heart failure at the time of randomization as well as during the 3 months of follow-up. At baseline and after 3 months of treatment, no differences with regard to safety parameters i.e. serum electrolytes, renal function, and oxidized low density lipoprotein levels were determined.

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**Table 1** Characteristics of study subjects and baseline medication

<table>
<thead>
<tr>
<th></th>
<th>Pravastatin + irbesartan (PRAV + IRB)</th>
<th>Pravastatin (PRAV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>30 (100%)</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Male/female</td>
<td>16 (53.3%)/14</td>
<td>16 (53.3%)/14</td>
</tr>
<tr>
<td>Age</td>
<td>60 ± 9</td>
<td>59 ± 10</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>28 ± 4</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>4 (13.3%)</td>
<td>2 (6.6%)</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>30 (100%)</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>29 (96.6%)</td>
<td>29 (96.6%)</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>17 (56.6%)</td>
<td>15 (50%)</td>
</tr>
<tr>
<td>Bisoprolol</td>
<td>10 (33.3%)</td>
<td>14 (46.6%)</td>
</tr>
<tr>
<td>Atenolol</td>
<td>2 (6.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Calcium(^{2+}) channel blockers</td>
<td>4 (13.3%)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Amlodipin</td>
<td>2 (6.6%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Nitrendipin</td>
<td>1 (3.3%)</td>
<td>2 (6.6%)</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>13 (43.3%)</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>13 (43.3%)</td>
<td>7 (23.3%)</td>
</tr>
</tbody>
</table>
liver enzymes, or leukocyte counts were observed between the groups (Table 2).

**Systolic blood pressure and cholesterol fractions**

Systolic and diastolic BP showed a moderate reduction after 3 months of therapy (systolic BP: PRAV + IRB 139 ± 15 to 123 ± 12 mmHg and PRAV 137 ± 15 to 126 ± 13 mmHg; diastolic BP: PRAV + IRB 82 ± 9 to 76 ± 5.5 mmHg and PRAV 81 ± 9 to 79 ± 12 mmHg). However, the differences between the groups did not reach statistical significance for the lowering of systolic BP [estimated treatment effect of between-group comparison: 4.66 mmHg (95% CI -0.25; 9.58); P = 0.0626] with only a statistically mentionable effect on diastolic BP [3.26 mmHg (95% CI 0.37; 6.08); P = 0.0277] when adjusted to baseline.

The decrease in serum LDL cholesterol (PRAV + IRB 123 ± 25 to 98 ± 23 mg/dL and PRAV 128 ± 22 to 108 ± 27 mg/dL) and the effect on HDL-C (PRAV + IRB 51 ± 14 to 50 ± 13 mg/dL and PRAV 50 ± 15 to 51 ± 14 mg/dL) were without statistically significant difference between the two groups when adjusted to baseline [estimated treatment effect of between-group comparison for LDL cholesterol 9.57 mg/dL (95% CI 3.35; 22.48); P = 0.1434 and for HDL cholesterol 1.89 mg/dL (95% CI -2.63; 6.41); P = 0.4057].

**Impact on high-sensitivity-C-reactive protein**

Baseline levels did not differ between the groups with a trend towards numerically higher levels in the PRAV + IRB group (P = 0.0568). After 3 months, there was no difference between the two treatment regimens in the reduction in hs-C-reactive protein [IRB + PRAV 5.21 ± 1.39 to 2.86 ± 0.58 mg/L vs. PRAV 3.26 ± 0.90 to 2.66 ± 0.61 mg/L; baseline-adjusted treatment effect of between-group comparison 0.28 mg/L (95% CI -0.85; 1.40); P = 0.6246] (Figure 3).

**Impact on secretory phospholipase A2 type IIA activity, secretory phospholipase A2 type IIA protein, and oxidized low density lipoprotein**

Baseline levels of sPLA₂ activity, sPLA₂ protein, and oxLDL were not significantly different.

The combined treatment with PRAV + IRB resulted in a reduction in circulating levels of sPLA₂ activity (IRB + PRAV 73.03 ± 11.6 to 60.01 ± 8.6 U/mL vs. PRAV 82.44 ± 7.7 to 87.64 ± 9.3 U/mL) and sPLA₂ protein (IRB + PRAV 4720 ± 1096 to 3524 ± 725 pg/mL vs. PRAV 5009 ± 1168 to 5555 ± 1480 pg/mL). There was a significant reduction in the primary endpoint sPLA₂ activity [baseline-adjusted treatment effect of between-group comparison: 22.88 U/mL (95% CI 7.23; 38.53); P = 0.0049] (Figure 4) and in a significant but not so pronounced reduction in sPLA₂ protein [1793.82 pg/mL (95% CI 322.87; 3264.74); P = 0.0177] (Figure 5).

There was also a reduction in oxLDL levels in the PRAV + IRB group (IRB + PRAV 60.18 ± 2.9 to 47.06 ± 1.9 U/L), whereas oxLDL levels determined in the PRAV treatment group remained nearly unchanged (PRAV 79.01 ± 7.7 to 78.04 ± 8.2 U/L). The effect in the PRAV + IRB group on oxLDL serum levels was also -significantly more profound when compared with PRAV treatment alone [baseline-adjusted treatment effect of between-group comparison 162.55 U/L (95% CI 45.15; 279.95); P = 0.0075] (Figure 6). Moreover, the effect on oxLDL was independent of changes in LDL cholesterol. In fact, the combined treatment decreased the oxidized LDL-to-LDL-cholesterol ratio (50.8 ± 18.9 U/mg to 49.4 ± 19.6 U/mg). Thus, the impact on oxidized LDL-to-LDL-cholesterol ratio changes was significantly more pronounced in the PRAV + IRB group (−1.4 U/mg vs. PRAV +14.4 U/mg; P = 0.0361).

**Table 2** Raw data (mean and standard deviation) for blood pressure and laboratory parameters of all study subjects within the groups at baseline and follow-up

<table>
<thead>
<tr>
<th></th>
<th>Pravastatin + irbesartan (PRAV + IRB)</th>
<th>Pravastatin (PRAV)</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>After 3 months</td>
</tr>
<tr>
<td>sPLA₂ activity (U/mL)</td>
<td>73.03 ± 11.6</td>
<td>60.01 ± 8.6</td>
</tr>
<tr>
<td>sPLA₂ protein (pg/mL)</td>
<td>4720 ± 1096</td>
<td>3524 ± 725</td>
</tr>
<tr>
<td>oxLDL (U/L)</td>
<td>60.18 ± 2.9</td>
<td>47.06 ± 1.9</td>
</tr>
<tr>
<td>hs-C-reactive protein (mg/L)</td>
<td>5.21 ± 1.39</td>
<td>2.86 ± 0.58</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>139 ± 15</td>
<td>123 ± 12</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82 ± 2</td>
<td>76 ± 5.5</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>123 ± 25</td>
<td>98 ± 23</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>51 ± 14</td>
<td>50 ± 13</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>85 ± 12.4</td>
<td>83 ± 12.9</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.5 ± 0.48</td>
<td>4.6 ± 0.48</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>142 ± 3.9</td>
<td>142 ± 2.7</td>
</tr>
<tr>
<td>Leukocytes (10⁹/µL)</td>
<td>7.8 ± 2.4</td>
<td>7.4 ± 1.5</td>
</tr>
</tbody>
</table>

BP: blood pressure.
Raw data for blood pressure and laboratory parameters at baseline and after 3 months of therapy are presented in Table 2. The estimated baseline-adjusted treatment effect obtained by ANCOVA and the 95% CI of the differences between the two groups for the primary and secondary endpoints after 3 months of therapy are summarized in Table 3.

**Discussion**

Here we report that only the adjunction of pravastatin and irbesartan reduced sPLA2-IIA activity, sPLA2-IIA protein concentration, and oxLDL levels in patients with CAD. Oxidative modifications of lipoproteins are considered as pivotal contributors to atherosclerotic plaque development and both sPLA2-IIA and Ang II via its AT1-receptor are involved in LDL peroxidation. In fact, modifications of LDL mediated by sPLA2 isoenzymes result in enhanced oxLDL uptake by macrophages and increased LDL affinity for proteoglycans, a critical step for LDL diffusion and deposition in the vessel wall. Recent experimental data further demonstrated a cross-talk between cytosolic phospholipase A2 (cPLA2) and sPLA2-IIA with regard to free radical release suggesting an additional mechanism through which sPLA2-IIA may enhance oxidative LDL modification. Early evidence for a potential role of Ang II in LDL oxidation was gathered by Keidar et al. who reported an increased propensity of LDL obtained from hypertensive patients to oxidative modification in comparison with LDL from normotensive subjects. Meanwhile, various interactions between Ang II and oxLDL have been demonstrated, including increased affinity of oxLDL to its scavenger receptor, enhanced oxLDL uptake by macrophages, and elevated intracellular lipid peroxidation in different animal models of atherosclerosis as well as in human vascular cells. A possible interaction between an activated RAS and phospholipases has been indicated by observations showing that inhibitors of phospholipase A2, C, and D substantially decrease Ang II-induced macrophage lipid peroxidation. In fact, our group recently reported that Ang II-dependent LDL oxidation may effectively be reduced by a specific inhibitor of active sPLA2-IIA. Moreover, treatment with the AT1-receptor antagonist irbesartan alone decreased sPLA2-IIA expression and sPLA2-IIA activity in vitro and in vivo in a small population of patients with documented CAD. Thus, Ang II-induced AT1-receptor activation may enhance oxidative LDL modifications via sPLA2-IIA and thereby facilitate atheroprosesssion (Figure 7). Based on these findings, we investigated the potential impact of a combined treatment with pravastatin and irbesartan on sPLA2-IIA and oxLDL in patients with CAD. We postulated that the addition of an AT1-receptor antagonist to the standard secondary prevention therapy with a statin might exert additional effects on sPLA2-IIA and LDL oxidation. We here report, that both treatment regimens comparably influenced blood pressure, LDL-cholesterol levels, and the acute-phase reactant hs-C-reactive protein. Interestingly, sPLA2 activity was intensively reduced in patients treated with the adjunction of pravastatin and irbesartan with a significant but not so pronounced reduction in sPLA2 protein. This is consistent with the observation of Mallat et al. that whole sPLA2 activity may have a better prognostic value than the sPLA2-protein level in patients with CAD.
with CAD. In fact, pravastatin alone tended to result in numerically enhanced circulating sPLA2-IIA protein and activity which could be related to interferon-γ-dependent signalling events. On the basis of our previous findings, which suggested sPLA2-IIA as a mediator of Ang II-dependent LDL oxidation, we also investigated the impact of both treatment regimens on oxLDL levels. Surprisingly, only the combination of pravastatin with irbesartan reduced this parameter, independent of changes in LDL cholesterol, whereas treatment...
with pravastatin alone did not influence oxLDL levels. These results are contrary to the findings of other groups, even though similar dosages were used. These diverging observations could be due to the fact that the impact of pravastatin on oxLDL levels in patients with CAD has not been investigated yet. In addition, the relatively short treatment period used in this study as well as only moderately increased oxLDL levels may have influenced the results obtained. Moreover, we cannot fully exclude that by using another statin a different effect on oxLDL could have been observed, although a recent review by Boehm et al. stated that statins have not been proven to possess differential potencies (regarding their ability to reduce cardiovascular events) if they are used in a dosage which results in similar LDL reductions. In this regard, the dosage used in the study presented is in line with the dosage used in large-scale clinical trials demonstrating the effectiveness of 40 mg/day pravastatin in the primary and secondary prevention of cardiovascular events.

**Study limitations**

Even though our observation is in line and consistent with previous experimental and pre-clinical reports, one of our limitations is the rather small number of patients at low risk. Therefore, larger double-blind, placebo-controlled trials are warranted in order to
confirm our findings. In addition, even though it would be helpful to have a study arm without statin treatment, it is nowadays recommended in the guidelines for patients with CAD undergoing PCI to use statin treatment. Thus, we did not include a treatment group with AT1-receptor blockade alone. Such a group, however, would have helped us to clarify the individual impact of irbesartan on the parameters investigated under clinical conditions, even though our previous experimental work provided molecular insight into the potential underlying mechanism.

Finally, we cannot exclude an influence of some imbalance at baseline values because of our two patient dropouts in the PRAV group.

**Summary**

To summarize, we here demonstrate that only the combined treatment of irbesartan with pravastatin decreases the circulating levels of the cardiovascular prognostic marker sPLA2-IIA and of oxLDL in patients with CAD. This impact may, at least partially, be explained by the specific blockade of AT1-receptor-dependent sPLA2-IIA expression and activation, which results in the inhibition of sPLA2-IIA-induced LDL oxidation (Figure 7).

**Conflict of interest:** none declared.

**Funding**

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**References**


**Figure 7** Proposed mechanism for the impact of angiotensin II type 1-receptor blockade and statin treatment on secretory phospholipase A2-IIA and oxidized low density lipoprotein. Angiotensin II type 1-receptor blockade results in decreased formation and activation of secretory phospholipase A2-IIA. This decrease inhibits secretory phospholipase A2-IIA-dependent formation of leukotrienes and prostaglandins, resulting in less generation of reactive oxygen species and subsequently less oxidation of low density lipoprotein. Statin treatment directly reduces low density lipoprotein levels and oxidized low density lipoprotein formation. Thus, by targeting oxidized low density lipoprotein generation through different molecular mechanisms, angiotensin II type 1-receptor blockade and statin treatment may additionally influence atherosclerotic disease progression and, possibly, cardiovascular events.
24. Han WK, Sapirstein A, Hung CC, Alessandrini A, Bonventre JV. Cross-talk between cytosolic phospholipase A2 alpha (cPLA2 alpha) and secretory phospholipase A2 (sPLA2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2 alpha activity that is responsible for arachidonic acid release. J Biol Chem 2003;278:24153–24163.


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