Assessment of Patients' and Physicians' Compliance to an ACE Inhibitor Treatment Based on Urinary *N*-Acetyl Ser-Asp-Lys-Pro Determination in the Noninsulin-Dependent Diabetes, Hypertension, Microalbuminuria, Proteinuria, Cardiovascular Events, and Ramipril (DIABHYCAR) Study

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OBJECTIVE — The purpose of this study was to assess patients' and physicians' compliance with ACE inhibitor treatment, by measuring an endogenous biomarker of ACE inhibition, urinary *N*-acetyl-Ser-Asp-Lys-Pro (AcSDKP), in the Noninsulin-Dependent Diabetes, Hypertension, Microalbuminuria, Proteinuria, Cardiovascular Events, and Ramipril (DIABHYCAR) trial, which compared ramipril (1.25 mg o.d.) with placebo in 4,912 patients with type 2 diabetes and microalbuminuria/proteinuria.

RESEARCH DESIGN AND METHODS — The urine AcSDKP-to-creatinine ratio was measured blind to treatment in all participants who completed follow-up and provided spot urine samples ($n = 1,871$).

RESULTS — The median urinary AcSDKP-to-creatinine ratio was six times higher for ramipril than for placebo. Urinary AcSDKP-to-creatinine ratios displayed a bimodal distribution in both groups, with a very large intergroup overlap. Based on cluster analysis, we defined truly adherent ramipril patients as those with a ratio ≥4 nmol/mmol and truly adherent placebo patients as those with a ratio <4 nmol/mmol. After excluding patients withdrawing prematurely from the study or known to have used a nonstudy ACE inhibitor, 27.3% of the 597 ramipril patients had ratios <4, indicating poor compliance, and 9.7% of the 621 placebo patients had ratios ≥4, indicating intake of a nonstudy ACE inhibitor. Correcting for compliance by using AcSDKP-guided analysis affected surrogate outcome results (decrease in systolic blood pressure and urinary albumin excretion) only slightly.

CONCLUSIONS — The systematic use of spot urinary AcSDKP determination facilitated the detection of defects in compliance with ACE inhibitor treatment in both patients and physicians. Urinary AcSDKP measurement could be a useful biomarker for assessing compliance with ACE inhibition in the routine care of diabetic patients.

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The Noninsulin-Dependent Diabetes, Hypertension, Microalbuminuria, Proteinuria, Cardiovascular Events, and Ramipril (DIABHYCAR) trial conducted in proteinuric or microalbuminuric type 2 diabetes patients has concluded that a low daily dose of an ACE inhibitor (1.25 mg ramipril) had no statistically significant beneficial cardiovascular or renal effects over placebo after 4 years of follow-up (1). This result contrasted markedly with that of the Microalbuminuria Cardiovascular Renal Outcomes-Heart Outcomes Prevention Study (MICROHOPE) study, in which a high daily dose (10 mg) of ramipril administered in the evening was shown to be cardioprotective in microalbuminuric patients with type 2 diabetes after 4.5 years of follow-up (2). The eight times higher dose of ACE inhibitor used in the MICROHOPE study was considered the most likely explanation for the difference in results between the two trials, outlining the importance of selecting high doses rather than low doses of ACE inhibitors for cardiovascular protection. However, an unintended decrease in actual drug exposure because of poor compliance with treatment in patients given 1.25 mg ramipril would contribute to minimize a possible difference by comparison with a placebo. Indeed, a systematic review has

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Abbreviations: AcSDKP, *N*-acetyl-Ser-Asp-Lys-Pro; DIABHYCAR, Noninsulin-Dependent Diabetes, Hypertension, Microalbuminuria, Proteinuria, Cardiovascular Events, and Ramipril; MICROHOPE, Microalbuminuria Cardiovascular Renal Outcomes-Heart Outcomes Prevention Evaluation; SBP, systolic blood pressure; UAE: urinary albumin excretion.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Clinical and biological characteristics of the patient in the ramipril and placebo groups*

Intention-to-treat population	Ramipril	Placebo	*P* value
n	937	934	
Male sex	685 (73.1)	687 (73.6)	0.8262
Age (years)	64.5 ± 8.0	64.3 ± 7.8	0.6671
BMI (kg/m²)	29.5 ± 4.4	29.5 ± 4.7	0.9478
Fasting blood glucose (mmol/l)	9.5 ± 2.9	9.7 ± 3.1	0.1263
HbA_{1c} (%)	7.8 ± 1.7	7.8 ± 1.7	0.6860
Diabetes duration (years)	8 (4–15)	8 (4–14)	0.4197
SBP (mmHg)	144.4 ± 13.4	143.3 ± 13.0	0.0600
DBP (mmHg)	81.5 ± 8.4	81.2 ± 7.9	0.4050
Hypertensive	522 (55.7)	488 (52.2)	0.1331
Delay to start of hypertension treatment (years)	10 (5–15)	9 (5–15)	0.8554
Smoker (≥1 cigarette/day)	145 (17.7)	134 (16.7)	0.5719
Alcohol consumption (≥1 unit/day)	463 (55.3)	464 (55.7)	0.8740
Serum creatinine (μmol/l)	88.1 ± 18.2	88.1 ± 18.9	0.9827
Creatinine clearance (ml/min)	87.2 ± 27.1	88.5 ± 30.9	0.3250
UAE (mg/l)	65 (34–164)	66 (37–160)	0.4957
Microalbuminuria (20–200 mg/l)	738 (78.8)	741 (79.3)	0.7603
Proteinuria (≥200 μg/l)	199 (21.2)	193 (20.7)	0.7603
Previous retinopathy	54 (5.8)	42 (4.5)	0.2145
Previous cardiovascular disease	177 (18.9)	218 (23.3)	0.0183
Associated medication (no.)	4 (1, 10)	4 (1, 10)	0.7144

*Data are mean ± SD, *n* (%), median (interquartile range), or median (minimum, maximum). DBP, diastolic blood pressure.

shown that average adherence to oral hypoglycemic agents ranged from 36 to 93% in patients with type 2 diabetes (3). In the setting of a placebo-controlled randomized clinical trial, poor compliance to active treatment decreased effect size in an intention-to-treat analysis (4,5) and is very difficult to quantify (4), even if a clinical assessment of compliance with treatment is performed (4,6–8).

To assess patients' compliance with the ACE inhibitor treatment in the DIABHYCAR study, we investigated the use of a new endogenous biomarker, *N*-acetyl-Ser-Asp-Lys-Pro (AcSDKP) for monitoring the presence and magnitude of ACE inhibition. AcSDKP, a hemoregulatory peptide, is one of the substrates of ACE like angiotensin I (9). It is metabolized exclusively by ACE (9) and is excreted in urine (10). It had been shown to be a very sensitive marker of ACE inhibition (11) after the initiation of the MICROHOPE and DIABHYCAR studies. In small samples of patients, determination of AcSDKP in urine has provided a method for the detection of noncompliance with ACE inhibitor treatment with high sensitivity and specificity (10,12).

Results show that the systematic use of a spot urinary AcSDKP determination detected a 27.3% rate of noncompliance in a ramipril-treated group and a 9.7% rate of unscheduled ACE inhibitor prescription in the placebo-treated group. By comparison with the previously published results of the intention-to-treat analysis (1), correcting for compliance did not influence in a clinically significant way the results on blood pressure and urinary albumin excretion (UAE).

RESEARCH DESIGN AND METHODS

— The design of the study has been described elsewhere (1). DIABHYCAR was a 4-year, general practice–based, multicenter, randomized, double-blind, parallel-group trial comparing ramipril (1.25 mg o.d.) with placebo, in addition to usual antidiabetic and cardiovascular treatments, on the occurrence of cardiovascular and renal events in 4,912 patients with type 2 diabetes and persistent microalbuminuria or proteinuria.

Urine samples for the determination of AcSDKP and creatinine were obtained from all the 1,871 participants who completed follow-up and provided spot urine samples for UAE measurements. All AcSDKP determinations were performed blind to treatment using a commercially available enzyme immunoassay kit (AcSKDP EIA kit, SpiBio, CEA, Gif-sur-Yvette, France). The methods for UAE determination and the definition of significant albuminuria regression were as previously described (1).

Statistical methods

Frequency histograms and nonparametric clustering methods (13) were used to define an optimum threshold for detectable ACE inhibition, using the log-transformed urinary AcSDKP-to-creatinine ratio. The algorithm used for the clustering method was based on spherical uniform kernel density estimations and was implemented in the MODECLUS procedure of SAS statistical software (14). The validity of the cutoff point of the log-transformed urinary AcSDKP-to-creatinine ratio was checked, using various clustering algorithms and other methods, such as receiver operating characteristic curves (not shown).

Depending on the distribution of the variables, unpaired *t* tests or nonparametric tests were used to compare the ramipril and placebo groups. Categorical variables were compared using the χ^2 test. Covariance analysis, with treatment group and the baseline value as covariates, was used to assess the effect of treatment on systolic blood pressure (SBP) and UAE at last evaluation. The probability of UAE regression in the two treatment groups was compared by means of a logistic regression model. Continuous data are expressed as means ± SD or medians (interquartile range). AcSDKP-to-creatinine ratios are expressed as medians with 95% CI. A P value of < 0.05 was considered significant. Statistical analyses were performed with SAS statistical software version 8.2 (SAS Institute, Cary, NC).

RESULTS

Intention-to-treat population

Nine hundred thirty-seven patients were randomly assigned to the ramipril group and 934 to the placebo group; patients were similar on entry into the study for all factors except prevalence of previous cardiovascular disease, which was higher in the placebo group than in the ramipril group (Table 1). These patients define the intention-to-treat population. At randomization, 59% of the patients in the ramipril group and 57% of the patients in the placebo group were taking 4–10 additional nonstudy drugs. In the intention-to-treat population, 281 patients of the ramipril group (30%) and 234 patients of the placebo group (25.1%, $P = 0.017$) prematurely discontinued their assigned

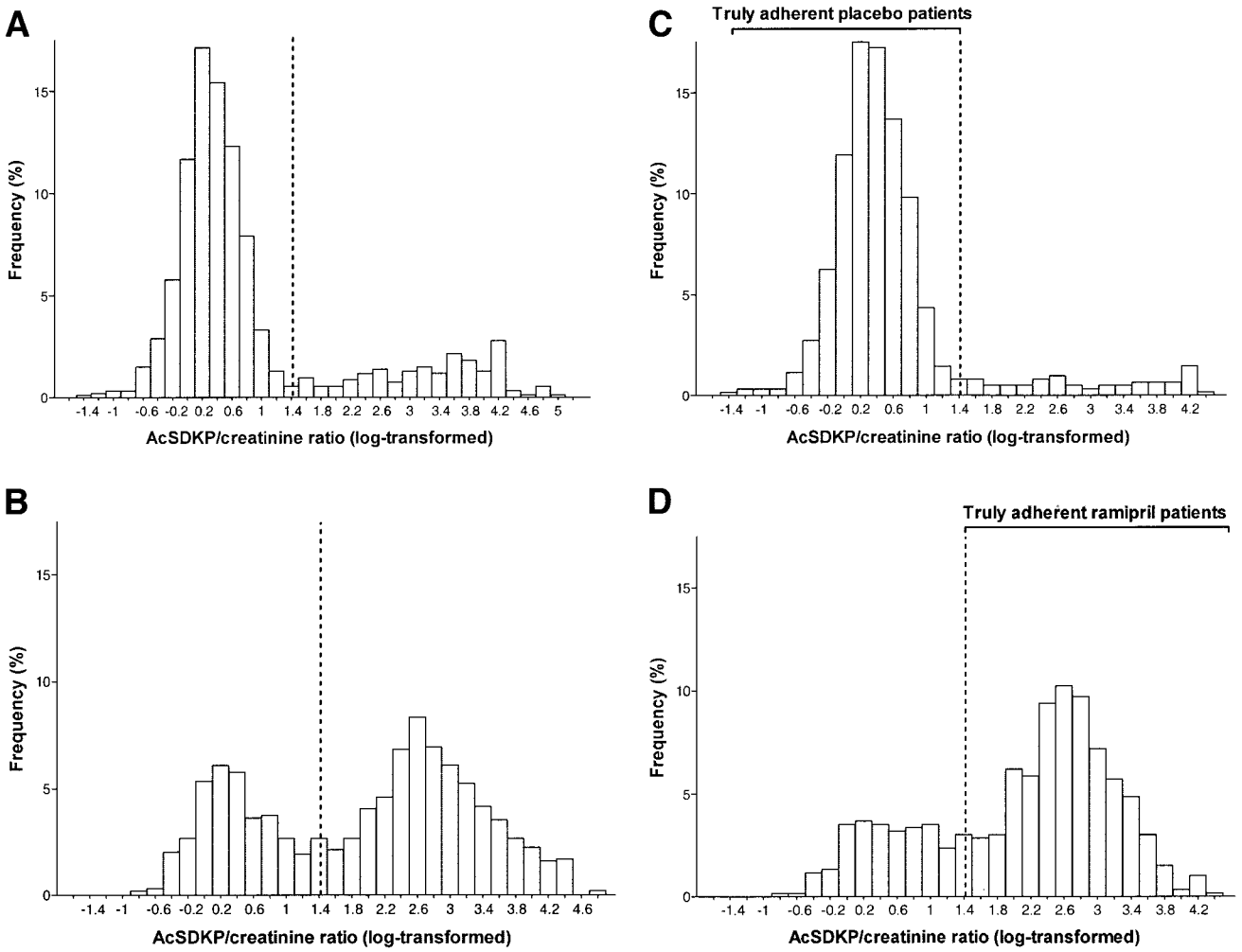

Figure 1—*Bimodal distribution of log-transformed AcSDKP-to-creatinine ratios in patients of the DIABHYCAR study.* A *and* B*: Intention-to-treat population with the placebo* (A; n = *934) and ramipril* (B; n = *937) groups.* C *and* D*: Per-protocol population with the placebo* (C; n = *597) and ramipril* (D; n = *621) groups. The dashed line shows the threshold of detectable ACE inhibition (1.38629 in log values, corresponding to 4 nmol/mmol), which defined* TA_P *and* TA_R *patients in the per-protocol population.*

double-blind treatment before the end of the trial because of either consent withdrawal or the occurrence of an adverse drug event, as reported in the case report form. In the intention-to-treat population, the prescription of a nonstudy open-label ACE inhibitor to 219 patients of the ramipril group (23.4%) and 212 patients of the placebo group (22.7%, $P = 0.73$) was reported in the case report form. Finally, 340 patients of the ramipril group (36.3%) and 313 patients of placebo group (33.5%, $P = 0.21$) had both conditions, i.e., premature discontinuation of their assigned double-blind treatment and open-label ACE inhibitor prescription.

Comparison of AcSDKP levels in the active and placebo groups

Intention-to-treat analysis. The AcSDKP-to-creatinine ratio achieved in the ramipril group was six times higher than that in the placebo group (median 9.67 [95% CI 8.78–10.93] vs. 1.54 [1.47–1.59] nmol/mmol, respectively, $P < 0.0001$). Log-transformed AcSDKP-to-creatinine ratio displayed a bimodal distribution in both the ramipril and the placebo groups (Fig. 1). There was a very large overlap of values between the two groups: the range of AcSDKP-to-creatinine ratios was 0.42–131 nmol/mmol in the placebo group and 0.26–141 nmol/mmol in the ramipril group (Fig. 1).

Per-protocol analysis. The same analysis was performed after excluding patients known to have prematurely discontinued their allocated double-blind treatment and those known to have been treated with an open-label ACE inhibitor, as reported in the case report form (ramipril group, $n = 340$ and placebo group, $n = 313$). This defines the per-protocol population ($n = 597$ in the ramipril group and $n = 621$ in the placebo group). This per-protocol analysis increased the difference in urine AcSDKP-to-creatinine ratio between the ramipril ($n = 597$) and placebo ($n = 621$) groups (ramipril median 10.82 [95% CI 9.62–11.78] vs. placebo 1.45 [1.38–1.54] nmol/mmol, $P < 0.0001$), but there was still a large overlap of values between the two groups (Fig. 1).

Evaluation of compliance with treatment in the DIABHYCAR study by urinary AcSDKP determination: determination of thresholds

Cluster analysis on log-transformed urinary AcSDKP-to-creatinine ratios confirmed the bimodal distribution of these ratios and delineated two separate clusters of patients in each group. We deter-

mined the threshold of detectable ACE inhibition by analyzing specifically the distribution of the AcSDKP-to-creatinine ratio in the cluster of patients treated with ramipril with high AcSDKP-to-creatinine ratios. Given the high sensitivity and specificity of urinary AcSDKP determination for assessing ACE inhibition (12), this cluster corresponds to patients who must have taken ramipril and/or a nonstudy ACE inhibitor. We selected a threshold of 4 nmol/mmol as the lower limit of the AcSDKP-to-creatinine ratio distribution in this cluster.

In intention-to-treat analysis, 331 of the 937 patients given ramipril (35.3%) had a ratio <4 nmol/mmol and 182 of the 934 (19.5%) patients given placebo had an AcSDKP-to-creatinine ratio ≥4 nmol/mmol. In the per-protocol analysis, 163 of the 597 patients in the ramipril group (27.3%) had a ratio <4 nmol/mmol and 60 of the 621 patients of the placebo group (9.7%) had a ratio ≥4 nmol/mmol. In the per-protocol population, we defined "truly adherent ramipril" patients (TA_R) as those with a ratio ≥4 nmol/mmol ($n = 434$) among the 597 patients of the ramipril group and "truly adherent placebo" patients (TA_P) as those with a ratio <4 nmol/mmol ($n = 561$) among the 621 patients of the placebo group (Fig. 1). The TA_R and TA_P groups had similar characteristics on inclusion (not shown).

Effects of ramipril on SBP and UAE, as determined by intention-to-treat, per-protocol, or AcSDKP-guided analysis

In intention-to-treat analysis, the baseline-adjusted mean difference in final SBP between the ramipril and placebo group was −1.40 mmHg (95%CI −2.55 to −0.25; $P = 0.017$). In per-protocol analysis, this difference was −1.73 mmHg (−3.06 to −0.40; $P = 0.011$). The AcSDKP-guided analysis slightly amplified the baseline-adjusted mean difference in final SBP between the TA_R and TA_P groups, from −1.40 mmHg (−2.55 to −0.25) to −2.23 mmHg (−3.69 to −0.77, $P < 0.0031$).

In intention-to-treat analysis, the proportion of patients with albuminuria regression in the ramipril group (27.1%, 254 of 937 patients) did not differ significantly from that in the placebo group (23.2%, 217/934 patients). Changes in UAE were also similar in the two groups (ramipril group median +3% [interquartile range −59 to 161] vs. placebo group +4% [−57 to 188], NS). In the per-protocol analysis, changes in UAE were also similar (ramipril group +3% [−59 to 153] vs. placebo group +9% [−56 to 196], NS). In AcSDKP-guided analysis, 120 of 434 TA_R patients (27.7%) presented significant regression of albuminuria vs. 124 of 561 TA_P patients (22.1%, $P = 0.044$). UAE regressed in patients receiving 1.25 mg ramipril (−7% [−60 to 143]) whereas it increased in patients taking placebo (+9% [−55 to 188], $P = 0.048$).

CONCLUSIONS — We report for the first time the use of an endogenous biomarker of compliance, urinary AcSDKP determination (10,12) in a large-scale, double-blind, randomized, placebo-controlled clinical trial of an ACE inhibition in patients with type 2 diabetes and microalbuminuria/proteinuria. At the end of this trial, the median AcSDKP-to-creatinine ratio was six times higher in the ramipril group than in the placebo group, overall confirming the fact that 1.25 mg ramipril o.d. effectively inhibited ACE. Interestingly, the distribution of the AcSKDP-to-creatinine ratio was bimodal in both the placebo and ramipril groups. Based on this bimodal distribution, we identified a AcSKDP-to-creatinine ratio of 4 nmol/mmol as the threshold defining detectable ACE inhibition. This threshold was used to define compliance with active treatment by patients and compliance with the protocol by physicians. Both were less than expected from the information collected in the case report forms. Because the AcSDKP methodology was not available during the DIABHYCAR study, the consequences of noncompliance, such as that objectively assessed by spot urine AcSDKP measurements, can only be analyzed on blood pressure and UAE in patients who had a complete follow-up. Correcting for compliance in these patients minimally affected these surrogate outcome results.

AcSDKP as a marker of noncompliance with treatment and/or the protocol

In the ramipril group, 35.3% of the patients had urinary AcSKDP-to-creatinine ratios <4 nmol/mmol in intention-to-treat analysis, within the range of values indicating an absence of ACE inhibitor intake. In the per-protocol analysis, 27.3% of the patients had AcSKDP-to-creatinine ratios less than the 4 nmol/mmol threshold, indicating a lack of compliance with ramipril treatment. This proportion may have been overestimated if some spot urine samples were collected >24 h after the last administration of the drug. However, the urinary AcSDKP-to-creatinine ratio remained high for up to 4 days after the cessation of treatment with an ACE inhibitor, such as 50 mg captopril b.i.d (10), and delays in urine collection with respect to the last drug intake are therefore unlikely to overestimate the percentage of nonadherent patients. We can conclude with confidence that those patients with a low AcSKDP-to-creatinine ratio did not take their dose of ramipril for at least a few days before urine sampling. Conversely, the proportion of ramipril patients with AcSDKP-to-creatinine ratios ≥4 nmol/mmol may overestimate compliance with daily treatment intake due to "white coat" or partial compliance (15,16), as the AcSDKP-to-creatinine ratio is extremely sensitive to ACE inhibition, increasing within 0.5–1 h after a single oral dose of 50 mg captopril (10).

Conventional clinical assessment methods estimated compliance to be similar in the DIABHYCAR and MICRO-HOPE trials. The percentage of patients in the ramipril group still receiving the allocated treatment at the end of the study, as assessed clinically by pill counting, was 70% in DIABHYCAR vs. 65% in MICRO-HOPE. However, compliance was not measured objectively at the time of publication of these results. Biological monitoring with urinary AcSDKP in DIABHYCAR gives less reassuring results than clinical monitoring. In large scale morbidity/mortality trials, such as DIABHYCAR or MICROHOPE, there may be considerable selective or nonselective noncompliance with the prescribed treatment for at least two reasons. *1*) Patients are already taking a large number of drugs (in DIABHYCAR, ~60% of the patients took 4–10 other, nonstudy drugs). This factor, together with the number of daily intakes, is known to decrease compliance (8). *2*) In such long-term randomized trials, patients may discard the experimental treatment (active or placebo) more readily than nonstudy drugs, as they are of unknown efficacy, as stated in informed consent forms (8). The ~25% noncompliance observed when considering AcSKDP-to-creatinine ratio is consistent with the level of noncompliance observed in diabetic subjects treated with oral hypoglycemic agents (15–33%) in prospective studies using electronic compliance monitoring systems (3).

In the placebo group, 60 of the 621 placebo-treated patients (9.7%) had AcSDKP-to-creatinine ratios ≥4 nmol/mmol, in the absence of any ACE inhibitor prescription explicitly mentioned in their case report form. This surprising finding highlights the magnitude of under-reporting of critical drug treatment by patients (who may be treated by several different physicians) or by physicians involved in the trial at least at its end.

The percentage of patients in the placebo group taking nonstudy ACE inhibitors, as assessed on the basis of interviews, was 22.7% in DIABHYCAR and 15% in MICROHOPE (2). A "cross-contamination" of the placebo group with the active drug is frequently observed in clinical trials especially *1*) in open-label trials (which was not the case for DIABHYCAR or MICROHOPE); *2*) if a biological/clinical marker of efficacy, such as plasma lipid concentration, can be easily monitored, as observed in the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) (17) and ALLHAT-LLT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) (18) trials; *3*) if the clinical condition of the patient deteriorates; or *4*) if the results of another clinical trial dealing with aspects of the disease under investigation are published before the end of the ongoing trial, with physicians anticipating the results of the new trial before publication (19). This may have been the case in the DIABHYCAR study, as the results of the MICROHOPE study were published on 22 January 2000 and DIABHYCAR ended on 21 March 2001 when urinary determinations were carried out.

Consequences of noncompliance and of the use of nonstudy ACE inhibitor

Noncompliance with the active treatment and the use of nonstudy ACE inhibitor in the placebo group in the DIABHYCAR and MICROHOPE studies would have minimized the magnitude of the beneficial effect of ramipril. However, correcting for compliance in the DIABHYCAR study makes only a small difference in surrogate outcome results, i.e., decreases in UAE and SBP. This finding reinforces the main conclusion obtained from the comparison of the two studies results: a strong inhibition of the renin-angiotensin system with high doses of an ACE inhibitor is required to reduce both cardiovascular risk and UAE in patients with type 2 diabetes and micro/macroalbuminuria. In the MICROHOPE study, the prescription of high doses of the ACE inhibitor may have contributed per se to minimize the issue of noncompliance with the active treatment. High doses produce more sustained and prolonged ACE inhibition (20) but also attenuate the effects of an intentionally or unintentionally missed dose (21), thereby reducing the impact of partial or noncompliance.

The routine use of urinary AcSDKP determination for monitoring ACE inhibition in clinical trials and clinical practice

There is no gold standard for measuring compliance with treatment. Direct determination of the plasma concentration of the drug is generally considered expensive, susceptible to white coat adherence bias, and complex (8). Urinary AcSDKP determination is inexpensive, based on a nonradioactive assay, and requires only a random spot urine sample at any moment of the day, with no special requirements for urine sampling. It can be performed by nonspecialist laboratories and does not require complex statistical models for its interpretation.

As shown in this large-scale ACE inhibition trial, the systematic use of this method could provide insight into patient noncompliance, which is underestimated by clinical methods, and compliance with the protocol, which is underestimated by the physicians' recording of nonstudy drugs in the case report form. These results confirm that the effect of poor compliance with active treatment is probably underestimated in most large-scale randomized controlled trials (22,23).

In usual care, compliance with treatment may be even poorer than in clinical studies, reducing the benefits of treatment below expectations based on trial results. Because ACE inhibition is a cost-effective therapy in patients with diabetes (24) and poor compliance to treatment is independently associated with poor clinical outcome (23), we suggest that urinary AcSDKP determination should be more widely used to detect noncompliance in patients with diabetes displaying a less favorable than expected response to ACE inhibitor prescription.

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