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The DIAB-HYCAR study

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Summary Microalbuminuria and proteinuria are strong independent predictors for increased cardiovascular mortality in non-insulin-dependent diabetic (NIDDM) patients. In such patients, angiotensin converting enzyme (ACE) inhibition improves the evolution of diabetic nephropathy; however, no data are currently available on the effects of such intervention on cardiovascular morbidity and mortality. The aim of the Diab-Hycar study is to test the hypothesis that ACE inhibition with a low daily dose of 1.25 mg ramipril, which has no significant effect on blood pressure, may reduce cardiovascular morbidity and/or mortality in normotensive or hypertensive NIDDM patients with persistent albuminuria. Selected and followed by general practitioners, 4000 patients will receive their usual oral antidiabetic treatment and if necessary antihypertensive treatment (ACE inhibitors excluded). In addition in a randomized, double-blind trial they will be given either a placebo or 1.25 mg ramipril daily. The follow-up is currently scheduled to last 3 years. The efficacy of ACE-inhibition will be assessed by the following major end-points: cardiovascular death, sudden death, myocardial infarction, stroke, renal replacement therapy. The Diab-Hycar study started on 3 February 1995. By 1 September 1995, 11 000 urine samples were tested. The prevalence of persistent albuminuria was 23 %, 964 patients were initially included in the study, with 619 eligible patients included soon after. Different strategies have been developed to record cardiovascular events correctly and to minimize the number of patients lost to follow-up. [Diabetologia (1996) 39: 1662–1667]

Keywords NIDDM, microalbuminuria, proteinuria, ACE-inhibition, cardiovascular morbidity and mortality.

Premature cardiovascular morbidity and mortality, mainly related to coronary heart disease (CHD) are major problems in non-insulin-dependent diabetic (NIDDM) patients. Different large-scale, prospective epidemiological studies have shown that in NIDDM patients the incidence of deaths from cardiovascular disease in males is double the incidence in matched non-diabetic subjects and is even higher in females [1]. In the Multiple Risk Factor Intervention Trial, NIDDM appeared to be a strong independent risk factor for CHD in males [2]. In NIDDM patients, microalbuminuria is not only a marker for the risk of developing diabetic nephropathy, but is also a strong independent predictor of increased mortality, mainly attributable to cardiovascular diseases.

Since the publication by Mogensen in 1984 [3], numerous studies have confirmed that microalbuminuria is associated with an increased incidence of cardiovascular deaths [4, 5]. Mac Leod et al. [6] have shown that this incidence also increased significantly in NIDDM patients with slightly elevated urinary albumin excretion, i.e. from 10.6 to 29.9 μg/min, a

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Abbreviations: INSERM, Institut National de la Santé et de la Recherche Médicale; CNAM, Caisse Nationale d'Assurance Maladie; ACE, angiotensin converting enzyme; CHD, coronary heart disease; NIDDM, non-insulin-dependent diabetes mellitus; CNIL, La Commission Informatique et Libertès.

range well beyond the common definition of microalbuminuria, compared to matched normoalbuminuric NIDDM patients.

Very few data are currently available on therapeutic interventions designed to reduce cardiovascular morbidity and mortality among NIDDM patients. No study has shown that improved glycaemic control has a long-term beneficial effect. In the double-blind, placebo-controlled study conducted by Ravid et al. [7] over a period of 5 years, angiotensin-converting enzyme (ACE) inhibition was observed to have a stabilizing effect on plasma creatinine and proteinuria in normotensive NIDDM patients. In a study by Lebovitz et al. [8] of 121 hypertensive NIDDM patients with various diabetic kidney diseases, 3 years of ACE inhibition was associated with a specific renal protective effect in addition to the lowering of blood pressure. Neither of these studies included any data concerning cardiovascular mortality. To our knowledge, no specific trial has been reported to investigate the effects of antihypertensive treatment, with or without ACE inhibition, on the morbidity or mortality of NIDDM patients.

However, during the last few years, different studies [9, 10] have shown that in patients with cardiac insufficiency or myocardial infarction and reduced ejection fraction, ACE inhibition significantly reduced cardiovascular mortality, as well as the incidence of new coronary events. Finally, ACE inhibition may reduce left ventricular hypertrophy, which is associated with increased mortality in hypertensive patients [11].

Rationale for using 1.25 mg ramipril daily. Ramipril is an effective and safe ACE inhibitor, currently used worldwide at daily doses ranging from 1.25 to 10 mg. It has no undesirable metabolic side effects in NIDDM patients [12]. The Acute Infarction Ramipil Efficiency Study [13], a double-blind placebo controlled study of patients with recent onset myocardial infarction complicated by cardiac insufficiency, has shown that the use of ramipril was associated with a significant reduction in mortality. At the low dose of 1.25 mg daily, ramipril was shown to have no significant antihypertensive effects in animal models or humans [14].

In insulin-dependent diabetic patients with microalbuminuria, 1.25 mg ramipril daily reduced urinary albumin excretion [15], and in hypertensive non-diabetic patients treated with furosemide, the addition of 1.25 mg ramipril daily improved the regression of left ventricular hypertrophy [16]. Lastly, in a double-blind placebo controlled, cross-over study conducted during a 4-week period, it was demonstrated in eight NIDDM patients that 24 h after ingestion of 1.25 mg ramipril daily, this dose inhibited ACE activity as effectively as the dose of 5 mg determined in vitro by Cushman's method, and in vivo by the angiotensin II/angiotensin I ratio (unpublished data). In short, chronic administration of ramipril 1.25 mg once a day inhibits ACE activity for 24 h after the last intake; has no significant antihypertensive effect; and has few side effects. The selection of this low dose is very important when investigating any specific effect of ACE inhibition on cardiovascular morbidity and mortality, separately from its effect of lowering blood pressure.

Consequently, the aim of the Diab-Hycar Study, now under way is to test the hypothesis that once daily ACE inhibition with a low dose of 1.25 mg ramipril reduces morbidity and/or mortality in normotensive or hypertensive NIDDM patients with microalbuminuria or proteinuria.

I Feasibility study

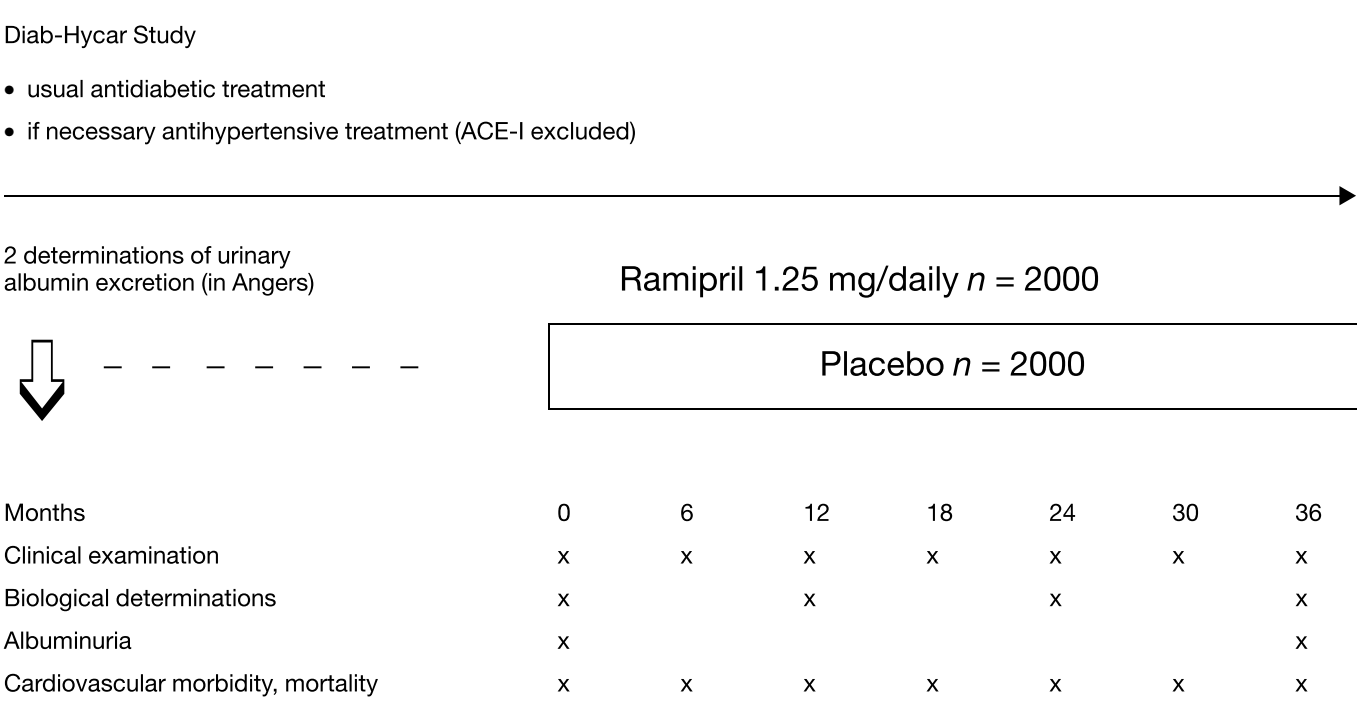
The final design of the protocol was preceded by a feasibility phase study [17]. During 1 month (1 November – 1December 1993) 792 general practitioners (GPs) were randomly selected and then contacted with the logistic support of Laboratoires Hoechst France. GPs were asked to provide Le Centre Hospitalier dÁngers with random urine samples from NIDDM patients, collected twice within 6 weeks; 509 GPs agreed to participate, and 326 worked actively and selected 2025 NIDDM patients whose urine was tested twice for albuminuria.

The prevalence of persistent microalbuminuria or proteinuria was 27.8 % in this population. The mean age of NIDDM patients with microalbuminuria or proteinuria was 64.3 ± 10.2 years.

In summary, about 30 % of the GPs contacted were active investigators and agreed to participate in a 3-year trial, and 80 % of the patients with albuminuria agreed to be involved in such a trial. These data were encouraging and useful for the design of the final protocol.

II Hypotheses and calculation of sample size for the trial

There was no direct way of calculating the number of subjects to be included in the Diab-Hycar study since there is no French cohort study on the risk of death of NIDDM patients with microalbuminuria. The expected rate of death was therefore based on a review of the available literature about the risk of death in patients with microalbuminuria and published cohort studies. Prospective studies of clinic populations (reviewed in [5]) and of population-based-cohorts [4–6, 18] have showed that the relative risk of early mortality in patients with NIDDM and microalbuminuria was 2.2 to 4.0 compared to NIDDM patients without microalbuminuria. In various cohorts of middle-aged



Months	0	6	12	18	24	30	36
Clinical examination	x	x	x	x	x	x	x
Biological determinations	x		x		x		x
Albuminuria	x						x
Cardiovascular morbidity, mortality	x	x	x	x	x	x	x

Fig. 1. see text

NIDDM patients (mean age 52–66 years), most without microalbuminuria followed from 3 to 6.1 years, the cardiovascular mortality ranged from 2 to 13 % [4–6, 18–21]. The low cardiovascular mortality rate in the Hypertension In Diabetes Study [19, 20] was explained by the inclusion of relatively young patients with recently discovered diabetes (less than 1 year). The high incidence in the ETDRS study [21] was due to the high proportion of patients with cardiovascular disease at inclusion. Taking into account these data and the characteristics of the expected population (most patients aged more than 60 years, frequently with hypertension and microalbuminuria), we anticipated a 12 % total mortality rate corresponding to a 6–7 % cardiovascular mortality rate and to a 20 % overall incidence of the principal endpoint (cardiovascular death, myocardial infarction, stroke and end-stage renal insufficiency).

The reduction in the number of these events in response to ramipril in NIDDM will probably reach a plateau due to the age of the subjects, as elderly patients may have other causes of cardiovascular or renal disease apart from diabetes. As a relative reduction in the risk of morbid events would not have any clinical relevance below a threshold of 20 %, this threshold was adopted for the calculation of sample size.

Assuming a 5 % α risk, 2000 subjects had to be included in each group to obtain a 90 % power of detecting a 20 % relative risk reduction. If the expected incidence of events is actually observed in the recruited population, 400 events will be observed in the placebo group, and 320 in the active treatment group.

III Protocol design (Fig. 1)

Selection of the GPs. From 1 October 1994 to 31 January 1995, 4000 general practitioners from across France were selected for their special interest in diabetes, hypertension or clinical trials. To be selected, they had to agree to recruit two NIDDM patients with microalbuminuria or proteinuria within 1 year, and to follow them up for 3 years. These patients would receive their usual antidiabetic treatment, if necessary an antihypertensive treatment (excluding ACE inhibitors as well as angiotensin II receptor antagonists). In addition, in a randomized, double-blind trial, they will be given either a placebo or 1.25 mg ramipril once a day. It was assumed that 50 % of the 4000 GPs would be active investigators, and would be able to include 4000 NIDDM patients with albuminuria or proteinuria in the trial.

Patients

Inclusion criteria: patients receiving oral antidiabetic agents (pragmatic definition of NIDDM)
– age 50 years or more, male or female
– with albuminuria ≥ 20 mg/l, determined on two occasions by immunoturbidimetry at the Centre Hospitalier d'Angers in random urine samples
– serum creatinine ≤ 150 μmol/l (Jaffe's method)
– normotensive or hypertensive
– giving of written, informed consent and agreement to provide the investigator with their present address and the addresses of two close relatives. The recording of this information was approved by La Commission Informatique et Libertés (CNIL, France).

Exclusion criteria: one of the following:
– treatment with insulin, an ACE inhibitor or an angiotensin II receptor antagonist
– urinary infection (Clinitek-dipstick test)
– cardiac insufficiency
– recent myocardial infarction, during the last 3 months
– previous intolerance to an ACE inhibitor
– chronic alcoholism or drug abuse, and inability to understand the methods and goal of the trial
– poor life expectancy (cancer, AIDS, etc.)
– refusal to sign the informed consent to participate in the trial.

Recording of clinical and biological parameters

At inclusion, the following clinical information is recorded:
– name, body weight, height, body mass index
– known duration of diabetes
– blood pressure, using a mercury sphygmomanometer with the patient in the recumbent position
– any current treatments
– cardiovascular risk factors: smoking habits, hyperlipidaemia, duration of hypertension, familial premature cardiovascular diseases
– previous cardiovascular complications: myocardial infarction, angina, cerebrovascular complications, arteritis, laser photo-coagulation for diabetic retinopathy
– and finally, a resting 12-lead ECG.

At inclusion, a 40 ml blood sample is collected, sent to the Centre Hospitalier d'Angers for DNA extraction, and stored with four aliquots of plasma and serum at –80 °C for determination of glycated haemoglobin, ACE activity, lipoproteins, etc.

At inclusion in the trial, the GPs provide the patients with the randomized treatment, i. e. placebo or ramipril, for a period of 6 months.

Consultations take place every 6 months and include a clinical examination similar to the initial one. Any modification in the treatment regimen is recorded, as well as undesirable side effects, the incidence of new pathology and cardiovascular complications.

Once a year, blood samples are collected for the determination of HbA_{1c}, serum creatinine and lipoproteins, and a random urine sample, for the determination of albuminuria. A resting ECG is also performed.

The follow-up is currently scheduled to last 3 years. The efficacy of ACE inhibition will be assessed on the following endpoints.

Major endpoints:
– cardiovascular death, sudden death
– documented myocardial infarction
– stroke
– renal replacement therapy.

Minor endpoints:
– death, whatever the cause
– hospitalization for coronary angioplasty or bypass revascularization
– documented cardiac insufficiency
– documented transient cerebrovascular ischaemia
– doubling of initial serum creatinine concentration
– loss of visual acuity in one eye
– peripheral amputation

Compliance with treatment
– compliance will be supervised by each investigator, who will record the cause and duration of treatment suspension; in cases of permanent cessation of treatment, data will be analysed for intention to treat.

This protocol was approved by the ethics committee of Centre Hospitalo-Universitaire d'Angers, and the study has been notified to the Medical Agency according to the current national regulations.

IV Study organization and functioning of the committees

This study is being carried out under the control of several committees whose functions are here described:

Scientific Steering Committee
• Finalization and validation of the protocol and case report forms for the principal study, and development and evaluation of ancilliary study protocols which must be specifically financed.
• Development of educational material designed for the training of investigators, followed by the publication of the study journal designed to maintain motivation throughout the study.
• In accordance with proposals of the Efficacy and Safety Surveillance Committee, the Scientific Steering Committee may prematurely suspend the trial, prolong the observation period or readjust the calculation of the sample size.
• Validation of analyses of the results of the study.
• Appointment of editorial committees composed of members of the other study committees most able to provide relevant information and actively participate in the preparation of publications concerning the study (its methodology, state of progress, principal and secondary results, ancillary studies, etc). Appointment of the authors of publications, giving preference to members of the editorial committees and participants in the ancillary studies, and relations with the medical press in general.
• Relations with the sponsor and health authorities (INSERM, CNAM, Drug Agency, French Ministry of

Health, Ministry of Research) and scientific societies (European and French Diabetology, Cardiology, Hypertension, Therapeutic and Nephrology societies).

Coordination and Logistics Committee
- Design and management of random treatment allocation by Minitel or vocal file server.
- Allocation of initial supplies to investigators, followed by the monitoring of subsequent drug supplies ensured by the Production Unit of Laboratoires Hoechst.
- Data collection by recording, data entry, and verification of detachable pages of the case-report forms, which will allow real-time constitution of the file used to analyse the results of the study.
- Telephone and on-site monitoring of serious events and/or events constituting part of the morbidity-mortality criterion.
- Audit of at least 10 % of the centres to ensure conformity with Good Clinical Practice.
- Submission of all serious events and/or events constituting part of the morbidity-mortality criterion to the Clinical Event Validation Committee.
- Closure of centres.
- Conduct of the analyses defined in the study protocol.

Critical Event Validation Committee
Appointed and supervised by the Scientific Steering Committee, this Committee, composed of independent experts, must validate all serious events and/or events possibly constituting part of the morbidity-mortality criterion.

Independent Patient Liaison Centre
The role of this centre is to investigate patients identified as being lost to follow-up by the investigators and by the Coordination and Logistics Committee.

Location of the Centre. Centre Régional de Pharmacovigilance de Saint-Etienne, Professor M. Ollagnier. C. H. R. U. Hôpital de Bellevue.

Clinical Pathology Committee. Responsible for the collection and storage of samples for laboratory tests, for conducting these tests, and for the sample bank of the study's laboratory.

Efficacy and Safety Surveillance Committee. Throughout the study, this committee will analyse the characteristics of the population of patients included, and the observed incidence of events corresponding to the assessment criteria for other events defined in the protocol. It will give its opinion concerning the validity of the calculation of the sample size, and the probability of being able to obtain a valid result.

It will also interpret the analyses of the intermediate reports on efficacy, safety and serious events. It will provide the Scientific Steering Committee with recommendations concerning the continuation, prolongation or suspension of the study.

V Preliminary recruitment data

The Diab-Hycar Study started on 3 February 1995. The study was announced at a Press Conference and a national meeting was held with 120 diabetologists, out of the 160 invited, who agreed to support a group of 20 to 30 local GPs. On account of the prevalence of microalbuminuria or proteinuria, in the population with NIDDM selected for the feasibility phase, it was estimated that urine samples from about 20000 NIDDM patients would have to be tested twice in order to include 4000 NIDDM patients with persistent albuminuria in the study.

By 1 September 1995, 11000 urine samples had been received in the Centre Hospitalier d'Angers. The prevalence of persistent albuminuria was 23 %, 964 NIDDM patients were included in the study, and 619 eligible NIDDM patients were included in the following weeks. This delay being due to summer vacations.

Of the GPs 1900 proved to be active investigators. Most of them have only tested one or two patients for albuminuria. A few of them tested up to 20 patients for albuminuria and included more than two patients in the study.

Discussion

The above data, obtained in less than 7 months, are impressive because French doctors and patients are not accustomed to participating in large intervention trials lasting 3 years. As it is currently estimated that the inclusion period will last for 20 to 24 months, the Scientific Steering Committee is developing new strategies to increase the rate of inclusion and to increase the number of investigators by 1500. We remain confident that 4000 NIDDM patients with albuminuria or proteinuria will eventually be included in the Diab-Hycar Study.

Another problem is to stimulate patients and GPs to comply strictly with the protocol for 3 years. A bimonthly issue of the Diab-Hycar letter is sent to all participants in the study (patients and investigators) to improve their knowledge of NIDDM, hypertension, diabetic angiopathy, clinical trials, etc, and to provide them with the current inclusion data. Once a year, all the co-ordinators, i. e. the diabetologists, will meet their active investigators for discussion and stimulation. They will have to deal with problems, such as some GPs being reluctant, for ethical reasons, to leave NIDDM patients who have albuminuria without ACE inhibition for 3 years despite the absence of scientific data indicating that this is harmful.

In such a study, the correct recording of endpoints is crucial. A full-time doctor is visiting hospitals, clinics and families to obtain all the necessary medical information. The number of patients lost to follow-up must be as small as possible – 0 being the optimal goal. To obtain this goal, two full-time technicians immediately start trying to locate any patient who misses a consultation by telephone and letter. They may also contact the relatives who agreed to give their address prior to the patient's inclusion.

The Diab-Hycar Study provides an opportunity to perform a clinical prospective genetic study of a large number of NIDDM patients at high risk of cardiovascular disease. It may be possible to correlate genotypes with patients' outcome and with the therapeutic effect of ramipril.

The Diab-Hycar Study may provide an answer to the crucial and still-debated question. Does long-term ACE inhibition reduce cardiovascular morbidity and mortality independently of its blood-pressure-lowering effect in NIDDM patients with albuminuria or proteinuria ?

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Composition of the different committees involved in the Diab-Hycar Study.

Scientific Steering Committee

F. Alhenc-Gelas, F. Cambien, J.P. Boissel, A. Girault-Louvel, P. Gueret, M. Marre (Principal Investigator), J. Menard (Vice Chairman), Ph. Passa (Chairman), P.F. Plouin, D. Vasmant (Secretary), Cl. Weisselberg.

Coordination and Logistics Committee

J.P. Boissel (Chairman), M. Marre, M. Lievre (Vice-Chairman), P.F. Plouin, J.C. Reglier (Secretary), D. Vasmant, F. Guenaneche.

Critical Event Validation Committee

G. Chatellier (Chairman), Th. Gabreau, Ph. Fressinaud, H. Leblanc, M. Azizi.

Clinical Pathology Committee

F. Alhenc-Gelas (Chairman), F. Cambien, A. Girault-Louvel (Vice-Chairman), M. Marre, J. Menard, M. Lievre.

Efficacy and Safety Surveillance Committee

E. Eschwege, S. Weber, N. Victor, C.E. Mogensen.

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