Combination Treatment With Metformin and Glibenclamide Versus Single-Drug Therapies in Type 2 Diabetes Mellitus: A Randomized, Double-Blind, Comparative Study

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To compare efficacy and tolerability of combination treatment with metformin and sulfonylurea with each of these drugs alone in the treatment of type 2 diabetes, 88 type 2 diabetic subjects (hemoglobin A_{1c} [HbA_{1c}] levels, 8.0% ± 1.0%; age, 57.3 ± 7.1 years; body mass index [BMI]. 27.0 ± 2.6 kg/m^2; diabetes duration, 9.8 ± 8.2 years; means ± SD) were randomly assigned to double-blind treatment with metformin (500 to 3,000 mg/d), glibenclamide (5 to 15 mg/d), or their combination (metformin 400 to 2,400 mg/d + glibenclamide 2.5 to 15 mg/d) for 6 months. Thereafter, groups were crossed over for the following 6 months. Thus, each patient received metformin or glibenclamide alone, and the combination treatment. Doses were titrated to obtain HbA_{1c} levels ≤ 6.0% and fasting plasma glucose levels less than 140 mg/dL. Eighty patients concluded both treatment periods and were included in the analysis of treatment efficacy. In patients receiving metformin or glibenclamide alone, the maximal dose was reached in 21 and 25 patients, respectively; 8 and 15 of these subjects, respectively, required the maximal dose when they were on the combination treatment. During the study, 4 (10.0%) subjects receiving metformin, 7 (17.1%) receiving glibenclamide, and 31 (39.2%) receiving the combination treatment reached HbA_{1c} levels ≤ 6.0%. Moreover, when efficacy of the combination treatment on glycemic control was compared with that of single-drug therapies in each individual patient, the combination was significantly more effective than either metformin or glibenclamide (HbA_{1c} after treatment, 6.1% ± 1.1% *v* 7.3% ± 1.4%, and 6.5% ± 0.7% *v* 7.6% ± 1.5%, respectively, both $P < .0001$). In conclusion, combination treatment with metformin and sulfonylurea is more effective than these drugs alone in improving glycemic control in type 2 diabetes, while also allowing a reduction of the dosage of each drug.

THE LARGE INTERVENTION trial, United Kingdom Prospective Diabetes Study (UKPDS), has recently demonstrated that also in type 2 diabetes complications may be significantly delayed or even prevented with strict glycemic control.[1,2] Initially, lifestyle modifications may be sufficient to achieve glycemic control in these subjects, but long-term adherence to such changes is unusual. Moreover, type 2 diabetes typically becomes more severe and difficult to treat over time. Thus, most patients require drug therapy with antihyperglycemic agents soon after the disease is diagnosed.

In most subjects with type 2 diabetes two defects coexist: defective insulin sensitivity and defective insulin secretion. Both of these abnormalities contribute to hyperglycemia.[3] Therefore oral therapy with either biguanide metformin, which improves sensitivity of peripheral tissues to insulin, or sulfonylureas, which stimulate insulin secretion, are rational approaches to type 2 diabetes mellitus. The UKPDS[1,2] has also shown that monotherapy with these oral agents often fails to maintain glycemic control over time, and many patients have to be switched to treatment with combinations of oral agents or to insulin therapy.

However, to date only few controlled studies have assessed the advantages of combination therapy versus monotherapies in improving hyperglycemia. The majority of these studies have examined the effects of metformin addition in patients with secondary failure to sulfonylurea. Only one previous study has compared primary combination of metformin and sulfonylurea with single-drug therapies.[4] Given that combination therapy may allow lower dosages of each drug, this approach might also have advantages over monotherapy in terms of side effects.

In the present randomized, double-blind trial, efficacy and tolerability of metformin and glibenclamide given alone or in combination were compared in 88 type 2 diabetic patients, using a cross-over design.

PATIENTS AND METHODS

Subjects

Eighty-eight type 2 diabetic patients (56 males and 32 females) were recruited among the outpatients of our Division. All of them had fasting plasma glucose greater than 140 mg/dL and hemoglobin A_{1c} (HbA_{1c}) ≥ 6.3%. Insulin-treated patients and those with ketonuria, concurrent medical illness, severe diabetic complications, or severe cardiovascular, hepatic, renal, respiratory, or pancreatic diseases were excluded from the study. Ten patients (11.4%) had newly diagnosed type 2 diabetes mellitus. Among the remaining 78 subjects recruited in the study, 12 (13.6%) were on nonpharmacological treatment and 66 (75%) were on oral antidiabetic medications. Hypertension (treatment with antihypertensive drugs and/or blood pressure values ≥ 160/90 mm Hg) was recorded in 50% of subjects. In particular, 32 of the 44 hypertensive patients were treated with one or more of the following drugs: angiotensin enzyme-converting (ACE)-inhibitors, diuretics, calcium-antagonists, β-blockers, or α-blockers. Ten subjects were treated for hyperlipidemia with statins or fibrates, and 11 patients were given other drugs (xsalicylate, H2-antagonists, benzodiazepines, allopurinol, thyroxine, estradiol) for associated disorders. All patients gave their

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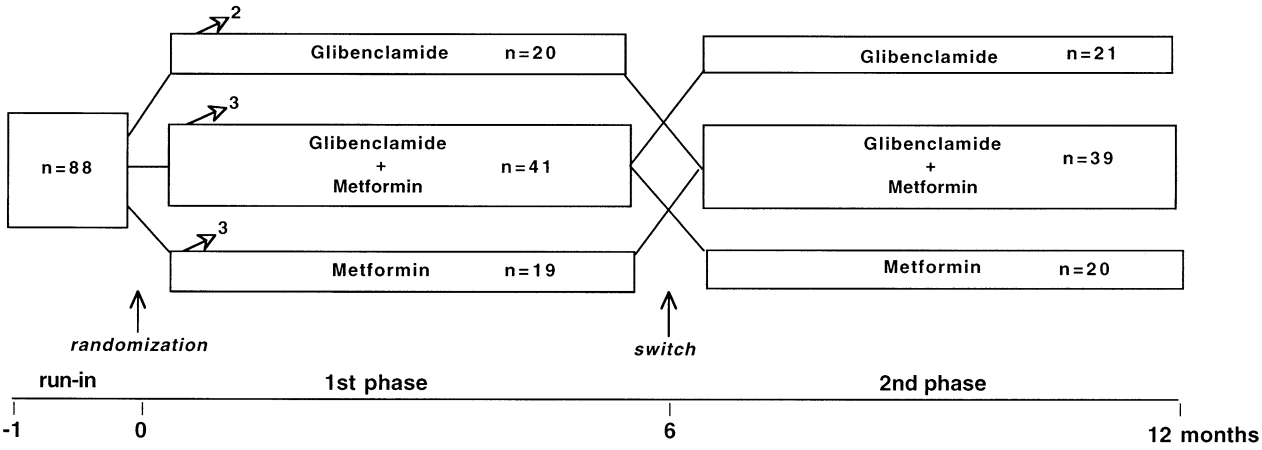

Fig 1. Study protocol.

written informed consent before entering the study, which was approved by the local Ethical Committee.

Study Design

Figure 1 summarizes the study design. At the recruitment visit (T −1), patients were instructed to follow a diet, which was individualized and kept constant thereafter. Previous treatments with oral antidiabetic drugs were discontinued. After a 4-week run-in period (T 0), eligible patients were randomized to 3 treatment groups (Fig 1), treated in a double-blind basis: group 1 (22 subjects) received glibenclamide alone, group 2 (22 subjects) received metformin alone, and group 3 (44 subjects) received metformin + glibenclamide. After 6 months all patients were crossed-over: subjects receiving monotherapies were switched to the combination treatment, whereas those receiving both drugs were switched to either glibenclamide or metformin alone. One patient randomized to receive the combination treatment and subsequently glibenclamide alone did not fulfil the inclusion criteria and was excluded from the study. As this patient was not replaced, 87 subjects received the study drugs.

During both phases of the study, doses were titrated in four steps (at intervals of minimum 20 days) to achieve $HbA_{1c} \leq 6.0\%$ and fasting plasma glucose less than 140 mg/dL, in the absence of hypoglycemic episodes. To this purpose, patients were asked when possible to monitor their daily profiles with a glucometer.

The starting dose was 1 tablet before lunch, consisting of glibenclamide 5 mg, metformin 500 mg, or glibenclamide 2.5 mg + metformin 400 mg. The subsequent steps were 1 tablet twice daily (before breakfast and before dinner), 2 tablets twice daily (before breakfast and before dinner), and 2 tablets three times daily (before breakfast, before lunch, and before dinner). For the group treated with glibenclamide alone, the last 2 steps were 1 tablet of active drug + 1 tablet of placebo taken together twice daily in the third step or 3 times daily in the fourth step. Therefore scheduled dose steps were 5, 10, 10, 15 mg/d for glibenclamide, 500, 1,000, 2,000, 3,000 mg/d for metformin, and 2.5 + 400, 5 + 800, 10 + 1,600, 15 + 2,400 mg/d for the combined glibenclamide + metformin.

All tablets were supplied by Guidotti Laboratories, Pisa, Italy, which generated the allocation schedule and provided the blinding procedure.

Clinical and Biochemical Evaluations

At the recruitment visit (T −1), patients provided a medical history and underwent a complete physical examination. The main measures of outcome, fasting plasma glucose, and HbA_{1c} levels were determined before randomization (T −1 and T 0) and, thereafter, every 2 months during both phases of treatment. At the same times, body weight, blood pressure, and heart rate were obtained, as well as information about treatment compliance, drug side effects, intercurrent medical events, and possible concomitant treatments.

Routine blood chemical tests, serum lipid profile (total and high-density lipoprotein [HDL]-cholesterol, triglycerides), creatinine clearance, fasting serum insulin, and plasma lactate were measured at baseline, after the run-in period, and at the end of each treatment period (T +6 and T +12), when patients were still on therapy. In addition, an electrocardiogram was recorded at the same time points.

Assays

HbA_{1c} was determined in duplicate by high-performance liquid chromatography, using a Hi-AUTO A_{1c} HA-8140 instrument (ARKRAY, KDK Corp, Kyoto, Japan) (reference interval, 3.0% to 5.5%). If a difference greater than 10% resulted between these assays, a third evaluation was performed and the mean of the 3 determinations considered. Plasma glucose was measured enzymatically with an automated analyzer (Beckman Instruments, Palo Alto, CA). Plasma insulin was measured by immunoradiometric assay, using a kit by Medgenix Diagnostics SA (Fleurus, Belgium). Plasma lactate was determined enzymatically with an ACA DUPONT analyzer (Wilmington, DE). Serum total cholesterol and triglycerides were measured using an enzymatic-colorimetric method (Boehringer Biochemia, Mannheim, Germany). HDL-cholesterol was determined by precipitation with phosphotungstic acid and magnesium ions (Boehringer Biochemia). Serum uric acid was measured by a commercial enzymatic method (Uricase-PAP), in an automatic analyzer.

Statistical Analysis and Calculations

Analyses of differences between treatments in quantitative variables of clinical efficacy (HbA_{1c} and fasting plasma glucose) were performed by analysis of variance (ANOVA), comparing the separate monotherapies and the combination therapy, after excluding any period effects or treatment-period interactions. In addition, differences in success/failure between treatments were assessed by the McNemar exact test. For this purpose, values at the end of each period of $HbA_{1c} \leq 6.0\%$ and fasting plasma glucose less than 140 mg/dL were considered as successes.

For the other parameters examined, treatment differences were assessed by ANOVA, after excluding any period effects or treatment-period interactions. As body mass index (BMI) changes showed a

significant period effect, comparisons between treatment groups for this variable were performed only in the first phase of the study.

To assess predictors of treatment efficacy, multiple regressions analyses were performed, including HbA_{1c} as dependent variable. Candidate predictive variables were age, sex, BMI, baseline metabolic features, diabetes duration, previous antidiabetic treatment, estimates of β-cell function, and insulin resistance.

BMI was calculated as body weight (kg)/squared height (m^2).

Low-density lipoportein (LDL)-cholesterol was calculated by the Friedewald formula.[5]

Derivate variables of glucose metabolism, based on the HOMA method,[6] were calculated as follows: pancreatic β-cell function = 20 × plasma insulin (mU/L)/(plasma glucose [mmol/L] − 3.5); insulin resistance = plasma insulin (mU/L) × plasma glucose (mmol/L)/22.5.

Data are shown as mean ± SD or median (interquartile range).

Table 1. Baseline Characteristics of Diabetic Patients Who Completed the Study in the Two Groups of Treatment

	Metformin/ Combination	Glibenclamide/ Combination
No. of subjects	39	41
Sex (M/F)	23/16	28/13
Age (yr)	57.8 ± 7.4	57.3 ± 7.2
BMI (kg/m^2)	27.0 ± 2.9	26.9 ± 2.5
Diabetes duration (yr)	9.9 (4-14)	10.4 (3.7-15.5)
Fasting plasma glucose (mg/dL)	221 (184-263)	239 (185-277)
Fasting insulin (mU/L)	9.7 (6.6-11.9)	10.6 (7.1-12.2)
HbA_{1c} (%)	7.8 (7.0-8.7)	8.2 (7.2-9.1)
β-cell function by HOMA	25.6 (14.2-39.9)	26.5 (12.2-33.6)
Insulin resistance by HOMA	5.3 (3.5-6.1)	6.0 (3.9-7.4)

NOTE. Means ± SD or, for not normally distributed variables, medians (interquartile range) are shown.

RESULTS

Tolerability

During the first phase of the study, 3 patients, 2 in the metformin group and 1 in the glibenclamide group, dropped out for causes not related to treatment. Another 3 patients dropped out for causes that were considered possibly related to treatment: 1 subject presented marked hyperglycemia with ketonuria, during treatment with metformin 2,000 mg/d; 1 subject reported hypoglycemia, while taking the lowest dose of glibenclamide; and 1 subject, taking the highest dose of glibenclamide + metformin, experienced persistent abdominal pain, constipation, and anorexia, which did not ameliorate after reducing the drug doses.

Nine patients, 6 treated with glibenclamide + metformin, 2 treated with metformin alone, and 1 treated with glibenclamide alone, reported mild symptoms suggestive of hypoglycemia, which disappeared after reduction of drug dosage. Diarrhea was reported by 1 patient treated with metformin alone, whereas gastrointestinal discomfort was reported by 1 patient given metformin alone and 1 patient given glibenclamide + metformin. These side effects were relieved by reducing the study drug dose.

Routine blood tests showed moderate increase of ALT (130 U/L; baseline value, 35 U/L; reference interval, 6 to 40 U/L) in 1 patient treated with glibenclamide (10 mg/d). This abnormality spontaneously disappeared within 3 months, without discontinuing the study drug. Moderate leucopenia with neutropenia, observed in a diabetic woman receiving glibenclamide (5 mg/d) + metformin (800 mg/d), resolved after discontinuation of concomitant antihypertensive drugs (lisinopril and hydrochlorotiazide).

No cardiovascular event was recorded during the study.

Glycemic Control and Other Parameters of Glucose Metabolism

Eighty of 81 patients who concluded both treatment periods were considered in the analysis of treatment efficacy. Table 1 reports the baseline clinical and biochemical characteristics of these subjects. One patient who concluded the study was excluded from this analysis, because she needed prolonged insulin therapy (for cholecystectomy) during the first phase of treatment—when receiving glibenclamide + metformin—and did not comply with dosage during the second phase—on metformin alone.

In 39 patients receiving metformin alone who completed the study, the mean dosage was 1,530 ± 613 mg/d and the maximal dose was reached in 21 patients (53.8%); only 8 of these subjects (20.5%) required the maximal dose when they were on the combination treatment. In 41 patients receiving glibenclamide alone, the mean dosage was 8.66 ± 2.40 mg/d and the maximal dose was reached in 25 subjects (61.0%); 15 of these subjects (36.6%) received the maximal dose when they were on the combination treatment. Mean dosages during the combined treatment in these groups were metformin 912 ± 465 and glibenclamide 5.70 ± 2.90 mg/d in 1 group, and metformin 1095 ± 506 and glibenclamide 6.84 ± 3.16 mg/d in the other.

Figure 2 shows HbA_{1c} and fasting plasma glucose mean values in the treatment groups during the entire study period. As a whole, combination therapy proved significantly more effective than either metformin (HbA_{1c}, 6.1% ± 1.1% *v* 7.3% ± 1.4%; fasting plasma glucose, 139 ± 35 *v* 174 ± 42 mg/dL; both $P < .0001$) or glibenclamide alone (HbA_{1c}, 6.5% ± 0.7% *v* 7.6% ± 1.5%; fasting plasma glucose, 147 ± 32 *v* 188 ± 49 mg/dL; both $P < .0001$).

Efficacy of the combination treatment on parameters of glycemic control was compared with those of single-drug therapies in terms of success or failure in each individual patient (Fig 3). Four (9.8%) subjects receiving metformin alone versus 21 (51.2%) receiving the combination treatment, and 7 (17.0%) subjects receiving glibenclamide versus 10 (24.4%) receiving the combination treatment reached HbA_{1c} levels ≤ 6.0% ($P < .01$ and $P > .1$, respectively, by the McNemar exact test). In the same treatment groups, 7 (17.5%) versus 19 (46.3%) and 7 (17.5%) versus 21 (51.2%) subjects, respectively, reached fasting plasma glucose levels less than 140 mg/dL ($P < .01$ and $P < .001$, respectively).

Pancreatic β-cell function, estimated by the HOMA method, was significantly higher after treatment with glibenclamide + metformin than after either metformin (69.5 ± 64.9 *v* 39.9 ± 23.1, $P = .004$) or glibenclamide alone (70.5 ± 63.8 *v* 52.3 ± 45.6, $P = .042$).

Similarly, insulin resistance, assessed by the same method,

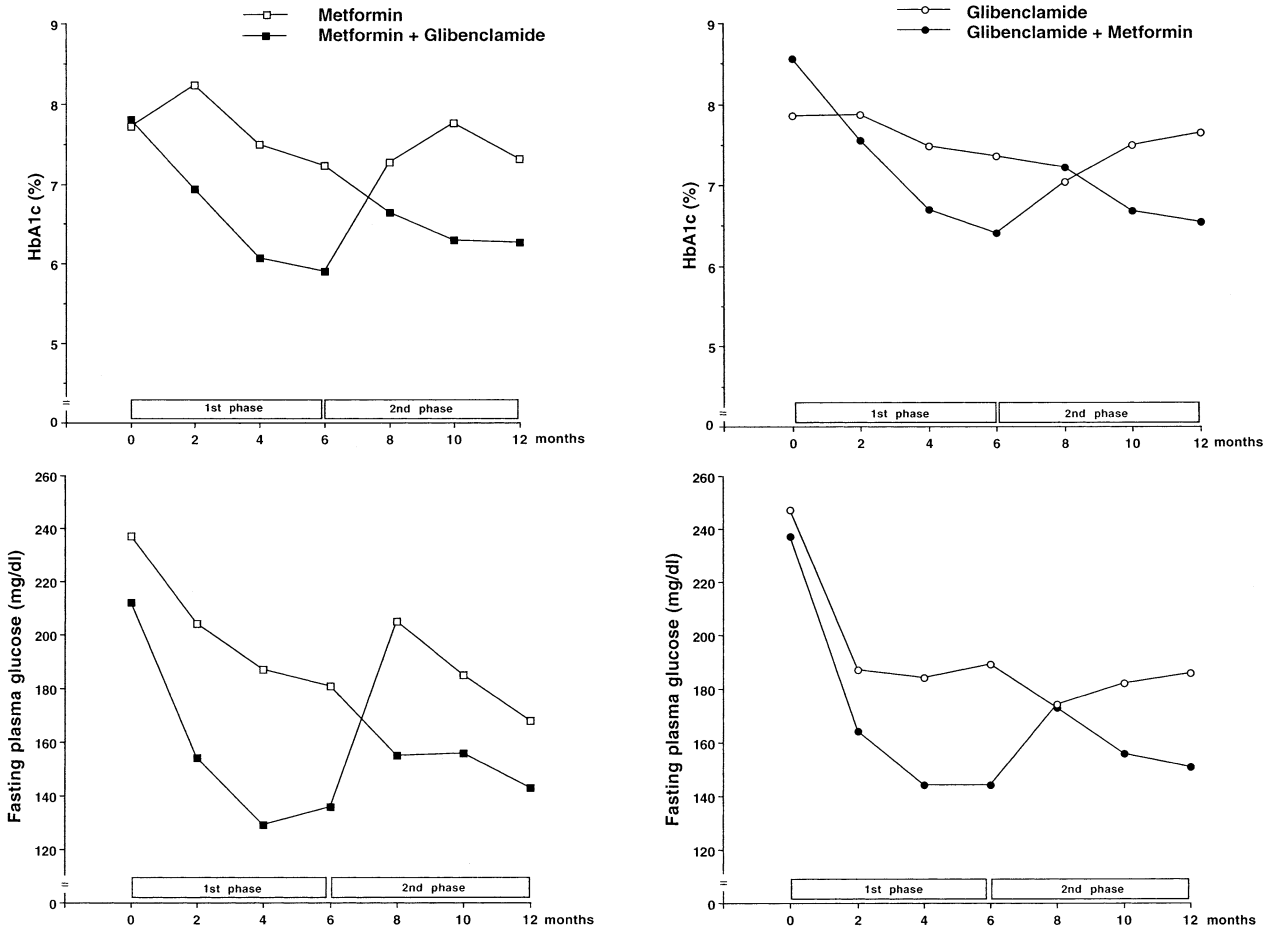

Fig 2. Comparison of HbA_{1c} and fasting plasma glucose levels in patients treated with metformin and metformin + glibenclamide (left panels) or glibenclamide and glibenclamide + metformin (right panels).

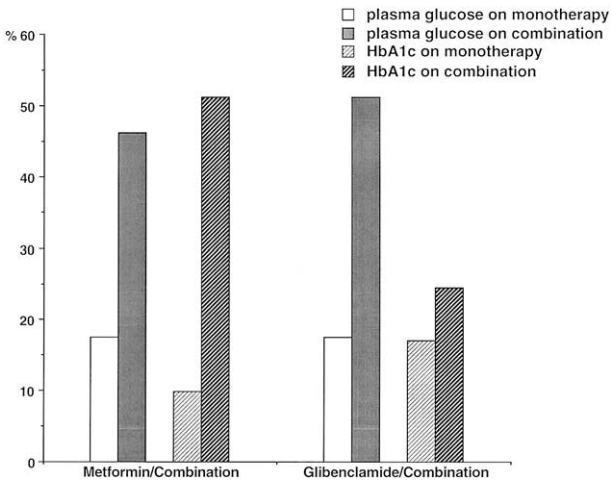

Fig 3. Comparison of percentages of success (fasting plasma glucose < 140 mg/dL, $HbA_{1c} \leq 6\%$) with monotherapies or combination in each treatment group.

showed a significantly greater improvement during the combination therapy than during either metformin or glibenclamide alone (3.9 ± 2.1 *v* 4.6 ± 2.4, $P = .014$; and 4.6 ± 3.3 *v* 6.4 ± 4.5, $P = .002$, respectively).

To analyze factors affecting efficacy of antidiabetic oral agents, multiple regressions were performed, separately for subjects receiving each treatment. In these models, HbA_{1c} change was included as a dependent variable; age, sex, BMI, baseline values of HbA_{1c}, diabetes duration, and HOMA-derived estimates of pancreatic β-cell function and insulin resistance were candidate predictive variables. Baseline HbA_{1c} was a predictor of drug efficacy in all treatment groups. In addition, HOMA-derived estimate of pancreatic β-cell function was a significant independent predictor of treatment efficacy with glibenclamide alone. Higher values of BMI increased metformin efficacy, but without reaching statistical significance. Finally, combined therapy efficacy decreased at increasing diabetes duration, with a borderline statistical significance ($P = .053$). When previous antidiabetic treament was included in the model as an additional independent variable, no significant effect was observed (data not shown).

Other Parameters

Due to a significant period-effect, comparisons among groups in BMI changes after treatment were performed only in the first phase of the study. At the end of this phase, BMI changes in patients given metformin alone (-0.51 ± 0.83 kg/m^2) were significantly different from those in patients receiving either glibenclamide alone or the combination therapy ($+0.27 \pm 0.88$ and $+0.23 \pm 1.10$ kg/m^2, respectively, both $P < .02$ *v* the metformin group).

Total and LDL-cholesterol levels were lower after the association treatment than after glibenclamide alone, whereas serum uric acid was higher after the association treatment than after glibenclamide. All treatments determined reductions of serum triglycerides, but no significant differences were found between treatment groups. Finally, no differences in plasma lactate levels were found between groups (data not shown).

DISCUSSION

The present controlled study demonstrated that the antihyperglycemic efficacy of combined glibenclamide and metformin was better than treatments with either drug alone. About 40% of patients treated with this combination achieved good glycemic control (ie, $HbA_{1c} \leq 6\%$), compared with only 10% to 17% of those treated with metformin or glibenclamide alone. Moreover, many patients given the combined treatment reached HbA_{1c} levels just above the target value. Remarkably, the mean absolute decline of HbA_{1c} was about 2% in diabetics treated with the combination, versus 0.5% obtained with each single drug. Consistent results were found by examining changes in fasting plasma glucose.

It is important to point out that, as expected, mean daily dose of each drug was substantially lower during the combined treatment than during the monotherapies (-40% for metformin, -31% for glibenclamide). Actually, about half of the patients who needed the maximal dosage during treatment with metformin or glibenclamide alone required the maximal dosage when given the combination treatment.

The synergic effect of the 2 drugs given together on improvement of glycemic control is consistent with the different mechanisms of action of the sulfonylureas (which mainly stimulate insulin secretion) and biguanides (which reduce hepatic glucose production and enhance insulin sensitivity). Differences between groups in changes after treatment of HOMA-derived estimates of pancreatic β-cell function and insulin resistance support this hypothesis. In this regard, our data also suggest that combination treatment is more effective than either drug alone in improving these parameters, probably because of the better glycemic control obtained with the combination treatment. In fact, hyperglycemia per se demonstrated adverse effects on both β-cell function and peripheral insulin sensitivity.[7,8]

In our study, side effects of antidiabetic drugs were infrequent, generally being mild and transient. In this regard, no significant differences were found between treatments. In most cases symptoms were promptly relieved by reducing doses of the study drugs.

Until now, only few controlled studies have addressed the efficacy of combination therapy versus monotherapy with sulfonylureas or biguanides. Most of these have assessed the effects of metformin addition in patients with nonoptimal glycemic control under sulfonylurea treatment.[9-12]

To our knowledge, only one other controlled study—presented in 2 complementary reports—compared biguanides, sulfonylureas, and their combination as a primary therapy.[4,13] Hermann et al randomized 144 type 2 diabetic patients to receive metformin, glibenclamide, or low-dose combination of these drugs for 6 months. Subjects from all groups who did not reach target fasting plasma glucose (120 mg/dL) within 6 weeks were switched to high-dose combination treatment. Target glycemic control was achieved in 62% to 66% of subjects given the monotherapies versus 75% of those given the low-dose combination therapy. Although this difference did not reach statistical significance, low-dose combination therapy was associated with lower weight gain than glibenclamide alone and fewer gastrointestinal symptoms than metformin alone. Moreover, the high-dose combination therapy was effective in many subjects who showed suboptimal glycemic control on monotherapies.

These findings are in agreement with the dose-response relationships known for hypoglycemic oral agents. About 75% of the hypoglycemic action of the sulfonylurea is usually observed with half the maximally effective daily dose.[14] Similarly, 80% to 85% of the maximal glucose-lowering effect of metformin is observed with 50% to 60% of the maximally effective daily dose.[14,15]

The design of Hermann's study allowed mainly short-term comparison of low-dose combination therapy with single-drug therapies. All subjects who did not achieve the blood glucose target early were given high-dose combination treatment. This may have reduced evidence of differences between treatments. In our study, subjects remained in the assigned treatment group for 6 months. Moreover, due to the crossover design, each subject received both the combination therapy and one of the monotherapies, allowing intrasubject comparisons. These characteristics of the protocol may explain why differences between treatments in glycemic control were more striking in our study.

As a whole, these considerations suggest that the commonly used option of increasing monotherapy to maximal dosage before adding a second drug might not be the best choice. Actually, low-dose primary combination therapy might be considered in most type 2 diabetic patients not adequately controlled on dietary treatment alone. However, some concern about combined therapy was recently raised by a substudy of UKPDS.[2] This study showed increased risk of diabetes-related and all-cause mortality after early addition of metformin in sulfonylurea-treated patients, compared with subjects who continued a sulfonylurea alone. This finding was somewhat surprising, as patients allocated to metformin alone showed a lower risk of any diabetes-related endpoint and all-cause-mortality. The same study also evaluated possible association of death from diabetes-related causes with the concurrent diabetes therapy, showing no increased risk in subjects treated with a combination of metformin and sulfonylurea. More recently, increased all-cause and cardiovascular mortality was reported in 169 diabetic patients taking the combination, as compared with 741 patients on sulphonylurea alone.[16] Moreover, increased all-cause mortality was reported in diabetic patients with chronic coronary disease receiving metformin, alone or in

combination with sulfonylureas.[17] However, in both these studies assignment to therapies was not randomized. It should be noted that we cannot rule out the possibility that in our study lack of adverse events with the combination therapy might be due to the short period of observation. This issue thus requires further study.

In conclusion, combination treatment with metformin and glibenclamide is more effective than each of these drugs alone in improving glycemic control in type 2 diabetes, also allowing a reduction of the dosage of each drug. These data would suggest that, in patients no longer responsive to low doses of monotherapy with sulfonylurea or metformin, the combination with low doses of a second oral agent is more advantageous than the maximal dose of a single drug.

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