Efficacy and Safety of HOE 901 Versus NPH Insulin in Patients With Type 1 Diabetes

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THE EUROPEAN STUDY GROUP OF HOE 901 IN TYPE 1 DIABETES

OBJECTIVE — HOE 901 (Hoechst Marion Roussel, Frankfurt, Germany) is a biosynthetic insulin with a prolonged action. The aim of this study was to compare the effect of the long-acting insulin analog HOE 901 with NPH insulin regarding glycemic control in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 333 type 1 diabetic patients were enrolled in this multinational parallel group trial. Subjects were randomized either to two different formulations of HOE 901 (the formulations differed only in zinc content) or to NPH insulin. The study was only partially blinded because patients can distinguish HOE 901 (a clear solution) from NPH (a cloudy suspension). In addition to premeal injections of regular insulin, patients received HOE 901 at bedtime or NPH once daily at bedtime or twice daily in the morning and at bedtime.

RESULTS — Fasting plasma glucose levels were significantly lower with HOE 901 (−1.88 mmol/l, $P = 0.0005$) as were fasting self-monitored blood glucose levels (−0.80 mmol/l, $P = 0.0020$). HbA_{1c} levels also showed a significant reduction with HOE 901 (−0.14%) versus NPH ($P = 0.030$). The overall frequency of hypoglycemia did not differ, but the frequency of nocturnal hypoglycemia was significantly ($P = 0.0037$) lower with HOE 901 (36 vs. 55%). However, this effect on nocturnal hypoglycemia was significant only versus NPH once daily, not NPH twice daily. The pattern of adverse events and injection site reactions with HOE 901 was similar to that with NPH.

CONCLUSIONS — This study indicates that HOE 901 achieves better control of fasting glucose and HbA_{1c} levels over 4 weeks, and HOE 901 has a possible safety benefit in terms of nocturnal hypoglycemia.

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The beneficial effects of achieving blood glucose values as close to the normal range as possible in type 1 diabetes have been firmly established (1). Still to be established is the best means of attaining a near-normal physiological pattern of insulin secretion and of improving life for patients by facilitating insulin therapy. Insulin profiles in healthy individuals are characterized by a relatively constant basal secretion with postprandial peaks. To satisfy the basal insulin requirement, one or more doses of intermediate or long-acting insulin are injected subcutaneously. However, the insulins currently on the market do not provide an appropriate basal supply (2). With NPH insulin, a maximal hypoglycemic effect is observed within 3–5 h after administration (3) and may lead to nocturnal hypoglycemia without providing adequate fasting blood glucose (FBG) concentrations because of the relatively short duration of action of NPH insulin. Long-acting insulins that do not have a peak action profile exhibit large intraindividual variations of absorption. With gene technology, attempts have been made to develop a long-acting analog with improved pharmacokinetic properties, particularly analogs designed for prolonged action without peaks and with lower day-to-day variation than the current protracted preparations (4–5).

The investigational drug HOE 901 is a new long-acting insulin analog with COOH-terminal elongation of the B-chain by two arginines and replacement of asparagine in position A21 by glycine (6). The resulting amino acid sequence (21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin) is illustrated in Fig. 1. The genetic information for 21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin is incorporated into *Escherichia coli* K12 via a safety plasmid. These modifications result in a shift of the isoelectric point from a pH of 5.4 toward a neutral pH, which makes the new molecule more soluble at a slightly acidic pH and less soluble at a physiological pH than the native insulin molecule. Because of this targeted protein design, HOE 901 precipitates locally in the subcutaneous tissue after injection, thus delaying its absorption and extending its duration of action. X-ray crystallography studies have demonstrated the importance of self-association for the biological properties of insulin analogs in vivo (7). The addition of zinc as a hexamer-stabilizing agent is expected to further prolong the duration of action of HOE 901. After confirmation of its biological activity in animal pharmacology studies in which it showed a delayed onset and a prolonged duration of action compared with NPH insulin, HOE 901 was administered to healthy volunteers (8,9) and type 1 diabetic patients (10). These studies revealed a much peakless profile and protracted action compared with NPH insulin. These spe-

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I.E.-J. and E.D. are employed by Hoechst Marion Roussel, a company that manufactures and markets insulin used in this study.

Abbreviations: ANCOVA, analysis of covariance; FBG, fasting blood glucose.

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

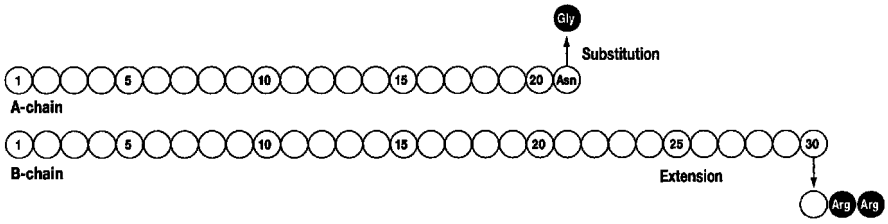

Figure 1—*Primary structure of HOE 901.*

cific pharmacokinetic properties suggest that HOE 901 is more suitable than NPH human insulin to mimic the normal pattern of physiological basal insulin secretion (11).

This phase 2 clinical trial was carried out in patients with type 1 diabetes to compare the 4-week efficacy and safety of two formulations of HOE 901 with NPH insulin, which is widely used as a basal insulin. The two HOE 901 formulations, HOE 901[30] and HOE 901[80], differ only in terms of their zinc content (30 and 80 µg/ml, respectively). Because animal studies showed an influence of the zinc concentration on the action profile, the study was performed to assist the decision regarding which of the two formulations of HOE 901 should be used in the phase 3 clinical trial. In addition, HOE 901 and NPH insulin were compared regarding their effect primarily on fasting plasma glucose and on other indicators of metabolic control.

RESEARCH DESIGN AND METHODS

Patients

After giving written informed consent, 333 patients with clinically diagnosed type 1 diabetes who had been receiving insulin therapy for >1 year were included in this study. The patient characteristics are shown in Table 1. A basal-bolus regimen of NPH insulin once daily at bedtime (n = 177) or twice daily in the morning and at bedtime (n = 156) plus regular human insulin before meals was used for at least 2 months. Exclusion criteria included the presence of known proliferative diabetic retinopathy, impaired hepatic or renal function, and a history of hypoglycemia unawareness.

Design

After a screening phase (7–14 days), patients were randomized to one of three treatment groups: HOE 901[30], HOE 901[80], or NPH insulin for the 4-week treatment phase. The patients in all three treatment groups additionally received regular human insulin before meals. Each of the 42 European centers included only once-daily or only twice-daily NPH patients. The two HOE 901 formulations (which are clear solutions) were compared under double-blind conditions, but the comparison between the HOE 901 formulations and NPH insulin (which is a cloudy suspension) could not be blinded. Bedtime insulin was injected into the abdomen between 2100 and 2300, and injection time was kept as stable as possible throughout the study. The first 3 weeks of the treatment phase were used to adjust the daily basal insulin dose according a titration scheme (FBG from 4 to 7 mmol/l without nocturnal hypoglycemia); basal insulin then was maintained during the final week of treatment. The dose of regular insulin was adjusted according the patients' habits, the premeal blood glucose concentration, and the carbohydrate content of the meal. The study was conducted in accordance with the Guidelines of Good Clinical Practice and the Declaration of Helsinki after approval by local ethics committees.

Clinical and laboratory determinations

Fasting plasma glucose, HbA_{1c} (reference range <6.5%), and fructosamine (reference range 200–278 µmol/l) levels were measured centrally at baseline and after 4 weeks. Home blood glucose monitoring (One-Touch II; LifeScan, Milpitas, CA) was used to derive the following variables: FBG recorded during the week preceding treatment and during the final week of treatment, the mean of a seven-point blood glucose profile, and nocturnal blood glucose at 0300 at baseline and at 4 weeks. Episodes of hypoglycemia (<2.8 mmol/l) were recorded by the patients and were classified as symptomatic, asymptomatic, and severe (requiring assistance). Hypoglycemia was reported as a serious adverse event when it led to coma or to a car accident. Antibodies to insulin were assessed by radioimmunoassay, and antibodies to *E. coli* were assessed by using immunoradiometric assay (Hoechst Marion Roussel, Frankfurt, Germany).

Statistical methods

The comparison of the two HOE 901 formulations (HOE 901[30] and HOE 901[80]) and the comparison of both HOE 901 formulations versus NPH insulin were performed by using a one-sided analysis of covariance (ANCOVA) (α = 10%) adjusted for baseline value and center effect. Descriptive statistics were used for baseline and safety data; for hypoglycemia descriptive statistics, a Cochran-Mantel-Haenszel test stratified by investigator and logistical regression was performed.

RESULTS

Fasting plasma glucose

The within-treatment changes in fasting plasma glucose from baseline to end point are shown in Table 2. Fasting plasma glucose decreased significantly within the HOE 901[30] and HOE 901[80] groups and remained stable within the NPH insulin group. The comparisons of the two HOE 901 formulations and of the pooled HOE 901 formulations and NPH are shown in Fig. 2. The adjusted mean values at the end of the study were similar (P = 0.27) for the two formulations (10.07 and 9.72 mmol/l for HOE 901[30] and HOE 901[80], respectively). When comparing the pooled formulations with NPH, a significant difference of 1.88 mmol/l (95% CI

Table 1—*Patient characteristics*

	HOE 901[30]	HOE 901[80]	NPH insulin
n	110	113	110
Male sex (*n* [%])	61 (56)	74 (66)	68 (62)
Age (years)	35.6 (18–68)	37.5 (19–70)	35.7 (20–61)
BMI (kg/m^2)	24.0 (18.7–28.3)	24.0 (18.6–30.3)	24.0 (18.9–29.1)
Duration of diabetes (years)*	11.0 (1.0–36.0)	8.0 (1.0–48.0)	11.0 (2.0–48.0)

Data are means (ranges) or *medians (ranges).

Table 2—*Description of main parameters by therapy*

	HOE 901 [30]	HOE 901 [80]	NPH insulin
Fasting plasma glucose (mmol/l)			
Baseline	12.76 ± 0.49 (98)	11.55 ± 0.42 (104)	11.91 ± 0.49 (98)
End point	10.57 ± 0.45 (98)	9.85 ± 0.42 (103)	11.92 ± 0.49 (98)
Change from baseline	−2.22 ± 0.58 (97)	−1.61 ± 0.46 (102)	0.01 ± 0.52 (98)
Within-treatment *P* value	0.0002	0.0007	0.98
FBG (mmol/l)			
Baseline	8.22 ± 0.22 (103)	7.97 ± 0.24 (103)	8.06 ± 0.25 (102)
End point	7.47 ± 0.20 (103)	7.18 ± 0.18 (101)	7.92 ± 0.28 (91)
Change from baseline	−0.73 ± 0.24 (98)	−0.80 ± 0.26 (94)	−0.02 ± 0.27 (89)
Within-treatment *P* value	0.0028	0.0033	0.95
HbA_{1c} (%)			
Baseline	8.09 ± 0.11 (110)	7.96 ± 0.11 (113)	7.85 ± 0.11 (110)
End point	7.85 ± 0.10 (110)	7.80 ± 0.10 (112)	7.79 ± 0.09 (109)
Change from baseline	−0.25 ± 0.05 (110)	−0.15 ± 0.05 (112)	−0.03 ± 0.05 (109)
Within-treatment *P* value	0.0001	0.0061	0.56
Fructosamine (μmol/l)			
Baseline	378.4 ± 5.6 (110)	369.1 ± 5.8 (111)	371.6 ± 5.3 (109)
End point	366.1 ± 5.4 (109)	355.4 ± 4.9 (113)	362.7 ± 4.6 (109)
Change from baseline	−12.3 ± 3.3 (109)	−12.6 ± 3.6 (111)	−7.6 ± 3.6 (108)
Within-treatment *P* value	0.0003	0.0008	0.0352

Data are means ± SE (*n*) change from baseline. A paired *t* test was used for the within-treatment comparisons. FBG concentration was estimated by using the trimmed mean of seven daily measurements obtained during the screening phase or the last 7 days of the maintenance phase. The trimmed mean was calculated from the five central values of the seven measurements (the two extreme values were eliminated for the sake of robustness).

0.84–2.93) (P = 0.0005) was obtained in favor of HOE 901.

Other indicators of glycemic control

In general, the pattern of results for self-monitored FBG was quite comparable with that obtained for the primary efficacy variable of fasting plasma glucose. FBG decreased significantly within the HOE 901[30] and HOE 901[80] groups and remained stable within the NPH insulin group (Table 2). Comparison of the adjusted mean at end point for the two HOE 901 formulations revealed no statistical difference (HOE 901[30] 7.20 mmol/l, HOE 901[80] 7.05 mmol/l; P = 0.62). The comparison between the pooled formulations of HOE 901 and NPH insulin was again statistically significant in favor of HOE 901, but the magnitude of this effect (0.80 [0.29–1.30] mmol/l) was smaller than that reported above for fasting plasma glucose (P = 0.0020). No treatment effect was detected in the case of the blood glucose profile and nocturnal blood glucose values at 0300. For all of these variables, no changes were evident within groups during the study.

Initial HbA_{1c} values of ~8% decreased significantly in both HOE 901 groups but remained stable in the NPH insulin group (Table 2). An overall treatment effect in favor of HOE 901 (P = 0.030) was evident. During the short study period of only 4 weeks, compared with NPH insulin, the adjusted mean HbA_{1c} at end point decreased by 0.17% in the HOE 901[30] group (P = 0.0087) and by 0.10% in the HOE 901[80] group (P = 0.10) (Table 3). The difference between the two HOE 901 groups was not significant. Fructosamine also showed a significant reduction in both HOE 901 groups from an initial value of ~370 μmol/l (reference range 200–278 μmol/l) and a slight decrease in the NPH insulin group (Table 2). In contrast with HbA_{1c}, no overall treatment effect was evident (P = 0.34), although the trend of the results was similar to that for HbA_{1c} (Table 3). Baseline values for both variables were different depending on the previous number of NPH insulin injections, but this observation did not hold true for glucose values. The group of patients already receiving NPH insulin twice daily had lower HbA_{1c} values (7.72 vs. 8.19%) and lower fructosamine values (359 vs. 385 μmol/l) than patients receiving NPH insulin once daily. From complementary analyses, this phenomenon did not seem to influence consistently the difference between treatments at the end of the study.

Hypoglycemia

A total of 256 patients (77%) reported at least one episode of symptomatic hypoglycemia during the 4-week treatment phase (Table 4). Symptomatic hypoglycemia was reported as a serious adverse event in two patients (one each taking HOE 901[30] and HOE 901[80]). One episode of symptomatic hypoglycemia in the NPH insulin group led to that subject's

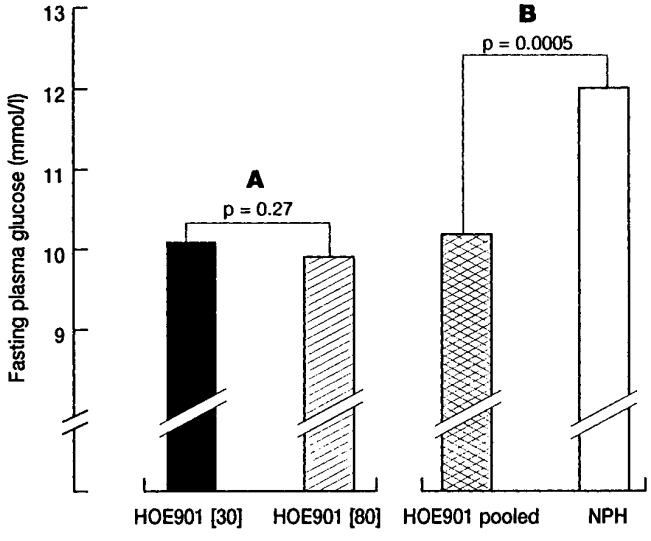

Figure 2—*The adjusted mean values of fasting plasma glucose at end point. Results of ANCOVA performed on HOE 901 patient data* (A) *and on the entire population* (B). ■, *HOE 901[30];* ▨, *HOE901[80];* ▩, *HOE 901 pooled;* □, *NPH.*

Table 3—*ANCOVA results for pairwise and global HbA_{1c} and fructosamine comparisons*

	Adjusted means at end point			Differences of adjusted means			
	HOE 901 [30]	HOE 901 [80]	NPH insulin	HOE 901[30] − NPH	HOE 901[80] − NPH	HOE 901[30] − HOE 901[80]	*P* for global comparison
HbA_{1c} (%)	7.71 (110)	7.77 (112)	7.88 (109)	−0.17	−0.10	−0.06	0.030
P				0.0087	0.10	0.31	
Fructosamine (μmol/l)	360.4 (109)	357.7 (111)	363.7 (108)	−3.3	−6.1	2.8	0.34
P				0.43	0.14	0.50	

Data are means (*n*). Pairwise treatment comparisons were performed for these variables by using the least square means and the contrasts derived from the ANCOVA. 95% CIs were calculated for each of the mean differences.

withdrawal from the study. Analysis of the number of patients with at least one episode of symptomatic nocturnal hypoglycemia during the entire treatment phase showed a statistically significant difference in favor of HOE 901 ($P = 0.0037$). However, the difference may depend on whether patients taking NPH insulin received one or two injections. HOE 901 appeared to have a clear advantage compared with NPH insulin once daily, but the total number of patients with nocturnal hypoglycemia was very similar when HOE 901 was compared with NPH insulin twice daily (Table 4). During the maintenance phase only, when nocturnal hypoglycemia was analyzed by using a logistical regression model adjusted for investigator effect and for nocturnal hypoglycemia during the screening phase, the overall treatment effect did not appear to be so unequivocal. The positive result during the maintenance phase may only relate to HOE 901[80] ($P = 0.0218$ vs. NPH) and not to HOE 901[30] ($P = 0.6249$ vs. NPH).

Insulin dose

The mean baseline dose of NPH insulin was 19 IU/day in patients treated with one injection a day and 23 IU/day in patients receiving two injections a day. In patients previously treated with one injection of NPH insulin a day and maintained on that regimen, an increase by 2 IU was evident in the dose of basal insulin in the HOE 901 groups, and a slight decrease by 0.5 IU was evident in the NPH insulin group ($P < 0.001$, HOE 901 vs. NPH). In patients previously treated with two injections of NPH insulin a day, the daily dose of basal insulin decreased by 4 IU in the two HOE 901 groups when they were transferred to one injection a day. Conversely, the daily dose of basal insulin increased slightly by 1 IU when they continued on two injections of NPH insulin a day ($P < 0.0001$, HOE 901 vs. NPH). For the daily dose of regular insulin (27 IU/day), no difference was observed between the three treatment groups.

Adverse events

The frequency and type of adverse events were similar for both HOE 901 formulations and NPH insulin. Injection site reactions were transient and comparable between groups and involved 3 patients (3%), 10 patients (9%), and 3 patients (3%) in the HOE 901[30], HOE 901[80], and NPH groups, respectively. No increase in HOE 901 insulin antibody titer or human insulin antibody titer was noted during the study in any of the treatment groups. An absence of effect was also noted for *E. coli* antibodies.

CONCLUSIONS

— The results of this study indicate that HOE 901 has a favorable benefit-to-risk assessment compared with NPH insulin in type 1 diabetic patients when administered as part of a basal-bolus regimen for a limited treatment period of 4 weeks. Both HOE 901 formulations (30 and 80 μg/ml) yielded similar results. These results support the decision to conduct all further studies with HOE

Table 4—*Patients with at least one episode of symptomatic, nocturnal, or severe hypoglycemia during the 4-week treatment phase and during the maintenance phase*

				NPH insulin subgroups		
	HOE 901[30]	HOE 901[80]	NPH insulin	Once daily	Twice daily	*P*
n	110	113	110	—	—	—
Total number of patients with hypoglycemia (4-week treatment)						
Symptomatic	87 (79)	82 (73)	87 (79)	49 (85)	38 (73)	0.5037*
Nocturnal	39 (36)	41 (36)	61 (56)	38 (66)	23 (44)	0.0037*
Severe	7 (6)	5 (4)	5 (5)	2 (3)	3 (6)	—
Total number of patients with hypoglycemia (maintenance phase)						
Symptomatic	44 (40)	32 (28)	47 (43)	27 (47)	20 (38)	0.0591†
Nocturnal	17 (15)	9 (8)	21 (19)	17 (29)	4 (8)	0.0218†
Severe	2 (2)	1 (1)	1 (1)	1 (2)	—	—

Data are *n* (%). *The Cochran-Mantel-Haenszel test was used to compare the three treatment groups (HOE 901[30], HOE 901[80], and NPH insulin); †adjusted pairwise comparison for HOE 901[80] and NPH insulin.

901[30]. Compared with NPH insulin, HOE 901 produced a highly significant reduction in fasting plasma glucose (−1.88 mmol/l). FBG was also significantly lowered in patients receiving HOE 901 compared with NPH insulin. The effect on FBG (−0.80 mmol/l difference between NPH insulin and the pooled HOE 901 treatment groups) was smaller in magnitude than the corresponding effect reported for fasting plasma glucose. However, the effects on both indicators were not expected to be identical. First, glucose values measured in venous plasma invariably differ from those measured in capillary blood. Second, the circumstances of measurement in the present study were different (patients measured glucose in capillary blood at home early in the morning just after waking). Venous blood samples for plasma glucose were withdrawn at the investigator site later in the morning during the clinical visit. In addition, a beneficial effect of HOE 901 on HbA_{1c} with a reduction of ~0.14% was observed, which is consistent with the results reported for fasting glucose determinations (12).

Overall, the incidence of hypoglycemic episodes was comparable for HOE 901 and NPH insulin. However, a significant difference in favor of HOE 901 in terms of symptomatic nocturnal episodes was evident but only for the treatment period as a whole. This difference involved HOE 901 versus NPH insulin once daily only. The analysis of hypoglycemic episodes was initially planned not only globally for the entire treatment period but also selectively for the final week of the study (i.e., the maintenance phase) when the dose of basal insulin was to be kept constant. The global positive effect of HOE 901 on the incidence of nocturnal hypoglycemic episodes was less obvious when attention focused only on the 1-week maintenance phase. A recognized tendency exists for patients to be more careful at the beginning of the treatment phase with a new treatment (i.e., HOE 901), and this phenomenon may explain the positive results when the treatment phase is considered as a whole. After stabilization, however, this study effect tends to disappear.

We found no systematic evidence that HOE 901 led to the development of insulin antibodies or *E. coli* antibodies. However, the study was too short to expect significant development of antibodies. The pattern of adverse and serious adverse events and injection site reactions with HOE 901 was similar to that with NPH insulin. Currently, preclinical data do not merit any special concern; IGF-I receptor–mediated growth-promoting activity of HOE 901 in muscle cells and the maximal metabolic activity of this analog are not different from those of native human insulin (13–14); IGF-I receptor signaling by HOE 901 is essentially identical to native insulin (15). HOE 901 also behaves like regular human insulin in terms of receptor binding, activation of the initial insulin-signaling chain, and promotion of mitogenesis (16). In addition, no carcinogenic effect has been observed in preclinical studies.

The interpretation of the comparison between HOE 901 and NPH insulin must be qualified because the comparison is based on a short-term open study and because the results obtained with NPH once daily and twice daily are different. In an open study, the change to the new insulin analog may cause patients to adhere to their treatment regimens more precisely and may account in part for the beneficial effects observed in fasting plasma glucose, FBG, and HbA_{1c}. As discussed above, the apparent advantage conferred by HOE 901 in terms of nocturnal hypoglycemia emerges only from the comparison with NPH insulin once daily.

The management of diabetes aims to eliminate hyperglycemia and reduce the risk of hypoglycemia. In addition, all attempts to improve the comfort of patients who need lifelong substitution therapy may be of great interest. A basal insulin injection once daily may help to improve the lifestyle of diabetic patients. The Diabetes Control and Complications Trial (1) demonstrated the benefit of strict glycemic control in delaying the development and progression of long-term complications. However, hypoglycemia limits effective intensive therapy (17,18). Methods to reduce the frequency of hypoglycemia, particularly at night, may be of a different nature (19). With existing insulins, the times and methods of administration may be changed. Recently, efforts have been made to design insulin analogs with an optimum action profile for overnight use, and HOE 901 is a candidate to match basal insulin supply requirements.

In conclusion, HOE 901 administered once daily at bedtime as a basal insulin in patients with type 1 diabetes was more effective than NPH insulin once or twice daily and was generally at least as safe as NPH insulin. Along with this global safety equivalence, a possible safety improvement may exist for HOE 901 versus NPH insulin once daily regarding nocturnal hypoglycemia. Based on the efficacy and safety results from the present study, HOE 901[30] and HOE 901[80] appear to be equally good candidates for further development.

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APPENDIX

The European study group of HOE 901 in type 1 diabetes

The following individuals and their site personnel participated in the European study group of HOE 901 in type 1 diabetes. Austria: T.R. Pieber, Graz; G. Biesenbach, Linz; R. Weitgasser, Salzburg; and K. Irsigler, Vienna. Denmark: K. Hermansen, Aarhus; C. Christiensen, Vejle; K. Soelling, Holbaek; H. Baekgaard Laursen, Kalundborg; and A.A. Prange, Kolding. Finland: M.R. Taskinen, Helsinki; T. Rönnemaa, Turku; E. Voutilainen, Kuopio; P. Salmela, Oulu; S. Bergkulla, Vaasa; and H. Haapamaki, Lahti. France: G. Charpentier, Corbeil Essonnes; and P. Valensi, Bondy. Germany: M. Haslbeck, München; H.U. Janka, Bremen; H.J. Lembcke, Braunschweig; K. Federlin, Giessen; W.A. Scherbaum, Leipzig; U.A. Müller, Jena; S. Matthaei, Hamburg; K.D. Palitzsch, Regensburg; K.H. Usadel, Frankfurt; M. Dreyer, Hamburg; W. Piehlmeier, R. Landgraf, München; and A. Liebl, München. The Netherlands: T.W. Van Haeften, Utrecht; J.B.L. Hoekstra, Utrecht; J. Van Hoogenhuijze, Leidschendam; B.H.R. Wolffenbuttel, Maastricht; and L.G. Van Doorn, Tilburg. Norway: S. Vaaler, Jessheim; I. Froeyshov Larsen, Oslo; R.K. Ganss, Nordbyhagen; and D. Dyrbekk, Toensberg. Sweden: S. Attvall, Goteborg; C. Berne, Uppsala; and E. Lins, Danderyd. Switzerland: P. Diem, Bern.

References

1. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. Binder C, Lauritzen T, Faber O, Pramming S: Insulin pharmacokinetics. *Diabetes Care* 7:188–199, 1984

3. Owens DR, Jones IR, Birtwell AJ, Burge CTR, Davies CJ, Heyburn P, Heding LG: Study of porcine and human isophane (NPH) insulins in normal subjects. *Diabetologia* 26:261–265, 1984
4. Markussen J, Diers I, Hougaard P, Langkjaer L, Norris K, Snel L, Sørensen AR, Sørensen E, Voigt HO: Soluble, prolonged-acting insulin derivatives. III. Degree of protraction, crystallizability and chemical stability of insulins substituted in positions A21, B13, B23, B27 and B30. *Protein Eng* 2:157–166, 1988
5. Brange J, Hansen JF, Langkjaer L, Markussen J, Ribel U, Sørensen AR: Insulin analogues with improved absorption characteristics. *Horm Metab Res* 26 (Suppl.): 125– 130, 1992
6. Seipke G, Geisen K, Neubauer HP, Pittius C: New insulin preparations with prolonged action profiles: A21-modified arginine insulins. *Diabetologia* 35 (Suppl. 1):A4, 1992
7. Hilgenfeld R, Dörschug M, Geisen K: Controlling insulin bioavailability by crystal contact engineering. *Diabetologia* 35 (Suppl. 1): A193, 1992
8. Dreyer M, Pein M, Schmidt C, Heidtmann B, Schlünzen M, Roßkamp R: Comparison of the pharmacokinetics/dynamics of Gly(A21)-Arg(B31, B32)-human insulin (HOE71GT) with NPH-insulin following subcutaneous injection by using euglycaemic clamp technique. *Diabetologia* 37 (Suppl. 1):A78, 1994
9. Coates PA, Mukherjee S, Luzio S, Srodinski KA, Kurzhals R, Roßkamp R: Pharmacokinetics of a "long-acting" human insulin analogue (HOE 901) in healthy subjects (Abstract). *Diabetes* 44:A130, 1995
10. Talaulicar M, Willms B, Roßkamp R: Efficacy of HOE 901 following subcutaneous injection for four days in type I diabetic subjects (Abstract). *Diabetologia* 37:A169, 1994
11. Pieber TR, Brunner GA, Siebenhofer A: Insulin analogues. In *Diabetes Annual.* Vol. 12. Marshall SM, Home PD, Rizza RA, Eds. Elsevier, 1999, p. 255–268
12. Pieber TR, Eugène-Jolchine I, Derobert E, for the European Study Group of HOE 901 in Type 1 Diabetes: Efficacy and safety of HOE 901 in patients with type 1 diabetes: a four-week randomized, NPH insulin-controlled trial (Abstract). *Diabetes* 47:A62, 1998
13. Bähr M, Kolter T, Seipke G, Eckel J: Growth promoting and metabolic activity of the human insulin analogue [Gly^{A21}, Arg^{B31}, Arg^{B32}]insulin (HOE 901) in muscle cells. *Eur J Pharmacol* 320:259–265, 1997
14. Eckel J, Kolter T, Bähr M, Lämmerhirt H, Spelleken M, Seipke G: Growth promoting and metabolic activity of the insulin analogue HOE 901 in muscle cells (Abstract). *Diabetologia* 38:A4, 1995
15. Liu L, Koenen M, Seipke G, Eckel J: IGF-I receptor-mediated signalling of the human insulin analogue HOE 901 (Abstract). *Dia - betologia* 40:A355, 1997
16. Berti L, Kellerer M, Bossenmaier B, Seffer E, Seipke G, Häring HU: The long acting human insulin analog HOE 901: characteristics of insulin signalling in comparison to Asp (B10) and regular insulin. *Horm Metab Res* 30:123–129, 1998
17. Diabetes Control and Complications Trial Research Group: Adverse events and their association with treatment regimens in the Diabetes Control and Complications Trial. *DiabetesCare* 18:1415–1427, 1995
18. Cryer PE: Hypoglycemia: the limiting factor in the management of IDDM. *Diabetes* 43: 1378–1389, 1994
19. Amiel S: Hypoglycaemia avoidance: technology and knowledge. *Lancet* 352:502–503, 1998