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HIM2

Treatment of Type 1 and Type 2 Diabetes
Insulin Analogue

Hexyl Insulin M2
Hexyl-Insulin Monoconjugate 2
NIN-058

α-Methyl-ω-hydroxypoly(oxy-1,2-ethanediyl) 29B-ether with 29B-[N^6-(6-hydroxy-1-oxohexyl)-L-lysine]insulin (human)

Modified recombinant human insulin by the conjugation of the oligomer methoxy(polyethylene glycol)hexanoic acid to the B29-Lys of human insulin

EN: 282659

CAS: 596796-49-5

Abstract

Currently available insulin preparations for the treatment of diabetes mellitus are unable to mimic the patterns of endogenous insulin secretion, and normoglycemia cannot be achieved. Inadequate control of hyperglycemia, particularly in patients with type 2 diabetes, is associated with severe microvascular and macrovascular complications. Thus, the search for improved insulin formulations that can reproduce the endogenous insulin secretion profile continues. Considerable advances have been made in the development of noninvasive insulin administration. Significant progress has been achieved in peroral insulin administration that could deliver insulin in a more physiological manner. One such orally active insulin is hexyl insulin monoconjugate 2 (HIM2), a single amphiphilic oligomer covalently linked via an amide bond to the free amino group on the Lys-B29 residue of recombinant human insulin. The alterations in the physicochemical properties of HIM2 as compared to unmodified insulin resulted in resistance to enzymatic degradation, facilitated absorption and reduced clearance from circulation. HIM2 has shown efficacy in improving postprandial glycemia and in insulinizing the liver in in vivo models, and has been shown to be safe and well tolerated, controlling postprandial plasma glucose levels in patients with type 1 and 2 diabetes.

Introduction

Diabetes mellitus is a chronic syndrome characterized by hyperglycemia, with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or action. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), 17 million Americans have diabetes and another 16 million suffer from impaired glucose tolerance, including insulin resistance syndrome. In 2000, the World Health Organization (WHO) reported that there were 154.4 million diabetics worldwide and predicted that by the year 2005, there will be almost 300 million people suffering from the disease (1-3).

Diabetes requires long-term drug therapy to limit and manage complications and to prevent premature death. Since the 1920s, insulin has been the standard of therapy for type 1 diabetes and is required in nearly half of all patients suffering from type 2 diabetes. The purpose of insulin therapy is to tightly control blood glucose levels and decrease progression of long-term complications. However, currently available insulin preparations are unable to mimic the patterns of endogenous insulin secretion, and normoglycemia cannot be achieved. Inadequate control of hyperglycemia, particularly in patients with type 2 diabetes, is associated with severe microvascular and macrovascular complications and tight control of fasting and postprandial glycemia in these patients has been shown to markedly reduce the incidence and progression of these complications (1, 4-10). Thus, the search for improved insulin formulations that can reproduce the endogenous insulin secretion profile continues.

Although considerable advances have been made in the development of injectable insulin analogues (11, 12), numerous daily injections of insulin are still required to sufficiently control postprandial glycemia. In addition, subcutaneous and parenteral insulin therapy can result in peripheral hyperinsulinemia, which is associated with

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coronary artery disease, hypertension, dyslipidemia and weight gain (13-16).

Excellent progress has been made in improving noninvasive methods of insulin administration (17, 18). Peroral insulin administration is one such noninvasive method that could deliver insulin in a more physiological manner. This method of delivery would not only improve compliance, but could reestablish the physiological ratio of portal vein to peripheral blood insulin concentration to approximately 1:5, as compared to about 0.75:1 for injectable insulin. This could provide more complete activation of the liver to participate in glucose metabolism. In turn, proper long-term activation of the insulin-dependent metabolic pathways of the liver could result in more optimal glycemic control and thus a decrease in the complications associated with diabetes. Until recently, the possibility of orally delivering insulin to be absorbed across the intestinal wall into the portal vein and produce sufficient, controlled antihyperglycemic effects has eluded researchers due to the instability of the insulins to enzymatic degradation. However, several efforts have been made to develop oral formulations of insulin which have proved to be safe and effective in slowing or preventing the progression of diabetes.

One such orally active insulin is hexyl insulin monoconjugate 2 (HIM2). HIM2 is composed of a single amphiphilic oligomer covalently linked via an amide bond to the free amino group on the Lys-B29 residue of recombinant human insulin (19, 20). When compared to unmodified insulin, the alterations in the physicochemical properties of HIM2 resulted in resistance to enzymatic degradation, facilitated absorption and reduced clearance from circulation. HIM2 has shown efficacy in improving postprandial glycemia and in insulinizing the liver. HIM2 was therefore chosen for further development as a treatment for type 1 and 2 diabetes (21).

Pharmacological Actions

Results from a rat hepatocyte insulin receptor binding assay showed that HIM2 bound to receptors with 67% the efficiency of unmodified human insulin. In addition, the agent was twice as resistant as insulin to chymotrypsin degradation. When administered to pancreatectomized dogs *in vivo*, 1000 μU/ml of insulin was detected in plasma 15 min postdosing with 1 mg/kg. In normal beagle dogs, the half-life of HIM2 was 5 times longer than that of insulin and its s.c. potency in mice was 80% that of insulin (20, 21).

An *in vitro* study examining the stability and physical characteristics of HIM2 showed that modification at Lys-B29 resulted in a change in the isoelectric constant as compared to human insulin (4.76 ± 0.2 *vs.* 5.51). This change suggests that the dissolution and solubility of HIM2 in the gastrointestinal tract would be improved, differing from unmodified insulin. The modification also increased the hydrodynamic radius of insulin (1.64 nm *vs.* 1.42 nm), although the self-association state of insulin at low protein concentrations was unchanged. HIM2 was also shown to be significantly more thermally stable than human insulin in aqueous buffer and in the solid state (22).

The polyethylene glycol (PEG) molecular distribution pattern of polydispersed HIM2 was isolated and identified. The PEG distribution ranged from PEG4 to PEG12, with PEG7, PEG8 and PEG9 accounting for about 70% of the polydispersed HIM2 composition. Biological potency of these separated PEG molecular weight forms of HIM2 exhibited similar biological activity in a mouse glucose assay (23).

Several other studies have reported the improved protease stability and solubility of HIM2 and have analyzed the conformational changes of the conjugate, showing no significant alterations in the biological activity of the parent insulin resulting from conjugation with the amphiphilic oligomer (24-26).

Oral administration of HIM2 (1.25 or 2.5 mg/kg p.o.) was shown to mimic the physiological pattern of insulin secretion into the portal vein, in contrast to recombinant human insulin (12.5 or 25 μg/kg s.c.) in a study using normal fasted CF-1 mice. Marked increases in portal vein insulin were observed in HIM2-treated mice by 15 min postadministration (up to 2789 μU/ml); these levels decreased to 221 μU/ml by 30 min postdosing. Vena cava insulin levels following HIM2 administration were 31-36% of those observed in the portal vein. On the other hand, s.c. administration of recombinant insulin resulted in peripheral insulin concentrations that were 2.4-6.9 times higher (up to 186 μU/ml at 15 min) than levels observed in the portal vein and a slower return to baseline levels (74 μU/ml at 30 min) was noted. However, HIM2 did not induce corresponding greater reductions in blood glucose as compared to recombinant insulin (31 and 23 mg/dl, respectively). These results suggest that HIM2 may bee less likely to induce hypoglycemia (27).

A study using fasted (42 h) normal dogs administered somatostatin (0.5 μg/kg/min) and basal amounts of insulin and glucagon intraportally at 4.5 h, and receiving a duodenal infusion of glucose at 15-270 min, reported that HIM2 (10 μU/kg/min pulsed over the first 5 min) was more effective than Humulin (human regular insulin; 10 μU/kg/min pulsed over the first 5 min) in activating the liver. Significant net hepatic glucose uptake was observed at 25 min in HIM2-treated animals as compared to 55 and 85 min for Humulin-treated animals and controls, respectively. Net hepatic glucose output at 60 min was –1 ± 1.04, 0.02 ± 0.66 and 1.21 ± 0.65 mg/kg/min for HIM2, Humulin and control groups, respectively. Nonhepatic glucose clearance was significantly higher in the HIM2-treated group (1.85 ± 0.15 ml/kg/min) as compared to controls (0.65 ± 0.29 ml/kg/min) and Humulin-treated (1.01 ± 0.18 ml/kg/min) animals. It was concluded that HIM2 was more effective due to its lower clearance,

which resulted in higher levels in both the periphery and liver (28).

Pharmacokinetics

A study has reported the optimization of an electrochemiluminescence assay to be used in the quantification of plasma insulin levels following oral administration of HIM2. The assay (ORIGEN®) was shown to be comparable to a validated HIM2 radioimmunoassay (RIA). The sensitivity and dynamic ranges of the ORIGEN® assay were 14.35 μU/ml and 14.35-2870 μU/ml, respectively (29).

A study using normal beagle dogs fasted overnight demonstrated dose-dependent absorption of an oil-in-water microemulsion of HIM2 (0.3, 0.6 and 1.2 mg/kg p.o.). Plasma glucose levels were dose-dependently lowered, with maximum reductions from baseline of 30%, 44% and 56% for the respective doses. Absorption was rapid, with C_{max} (30-237 μU/ml) achieved at 15 min postdosing (30).

The pharmacokinetics of i.v. HIM2 were shown to be improved over i.v. unmodified human recombinant insulin in a study in fasted beagle dogs. Animals were administered a single i.v. dose of either agent (3.75 μg/kg). The AUC obtained for HIM2 was twice as high as that for human recombinant insulin, while clearance was half that of human recombinant insulin. The calculated volume of distribution for HIM2 was 22% greater than that of human recombinant insulin and the plasma half-life was 2.4 times longer. Thus, the improved efficacy of HIM2 over unmodified insulin may be due to increased overall exposure to the agent (31).

Another study in beagle dogs examined the effects of gastric pH and emptying on the efficacy of liquid HIM2 formulations. Animals were administered oral HIM2 in high (pH > 8.0) or neutral pH formulations. Results obtained suggest that changes in gastric/duodenal pH may alter the rate of gastric emptying and oral absorption of HIM2 (32).

Both conjugated and unconjugated bile salts were shown to enhance the uptake of HIM2. HIM2 was formulated with various bile salts (at low concentrations relative to endogenous secretion levels) and administered to mice and dogs at doses of 2.5-5 and 0.5-1.0 mg/kg, respectively. The majority of bile salts examined enhanced absorption so that mean blood glucose was reduced 60-70% from baseline in both animals models; reductions were maintained for up to 2 h. Maximum reductions in dogs were observed at 15-30 min postdosing. Sodium salts including cholic, deoxycholic, glycodeoxycholic and taurodeoxycholic acids produced similar enhancing effects in both mice and dogs; ursodeoxycholate was less effective. The bile salts were effective at concentrations below the estimated critical micelle concentrations (CMCs) at the absorption site (33).

A randomized, crossover study conducted in 6 adult patients with type 1 diabetes receiving continuous s.c. insulin infusion therapy examined the timing of a standardized meal (50% carbohydrate, 30% fat, 20% protein) on the absorption of a single dose of HIM2 (0.25 mg/kg p.o. on 3 days 15 min before, immediately before or 5 min after a meal). Glucodynamic effects of the agent were observed following postprandial systemic exposure to glucose. When administered 15 min before a meal, HIM2 was rapidly and maximally absorbed. Absorption of the agent was decreased 79% and minimal when given immediately before and after a meal, respectively. The values for postprandial systemic exposure to glucose (AUC0-240) were 219.9 ± 360.5, 419.2 ± 231 and 465.9 ± 126.5 mg·h/dl for groups administered HIM2 15 min before, immediately before and after a meal, respectively (34).

Clinical Studies

A randomized, double-blind, placebo-controlled, crossover study conducted in 18 normal volunteers demonstrated the safety and efficacy of single escalating doses of HIM2 (0.3, 0.6, 1.2 and 2.4 mg/kg p.o.). All doses were well tolerated. Dose-dependent increases in serum insulin were observed starting at 15 min postdosing with 0.6 mg/kg or greater. Insulin levels 30 times greater (C_{max} = 74-283 μU/ml) than those observed in subjects treated with placebo were detected in peripheral blood in some of the subjects receiving HIM2. Blood glucose levels of 4 subjects receiving HIM2 were reduced to hypoglycemic levels and 2 of the subjects required rescue with a glucose infusion (35).

The efficacy of HIM2 was further demonstrated in a randomized, open-label study in 6 subjects fasted overnight and administered 3 doses of HIM2 (0.125, 0.5 and 075 mg/kg p.o.) on separate days, and another dose of HIM2 (0.5 mg/kg p.o.) and 2 s.c. doses of insulin lispro (0.1 U/kg) on separate days. At 60 min postdosing with 0.125, 0.5 and 0.75 mg/kg HIM2, fasting insulin levels increased maximally from 18 ± 2 μU/ml to 102, 321 and 561 μU/ml, respectively. Dose-dependent reductions in endogenous glucose production (primarily hepatic) and the rate of glucose disposal by peripheral tissue were observed starting 60 min posting with HIM2. The 0.5 mg/kg oral dose of HIM2 was shown to be comparable to 0.1 U/kg s.c. insulin lispro. Variability in the effects on the rate of glucose disposal were similar for both agents (25 ± 7% and 27 ± 1% for HIM2 and insulin lispro, respectively) (36). The results of this study and two of the following studies are summarized in Table I.

A study conducted in 16 patients with type 1 diabetes (daily insulin requirement = 27-60 U; glycosylated hemoglobin levels = 5.8-11.1%) fasted overnight confirmed the safety and efficacy of HIM2 (0.6, 0.8 or 1 mg/kg p.o. in the morning 30 min after discontinuation of i.v. insulin, followed by a second dose 120 min later). HIM2 was safe and well tolerated and no symptomatic hypoglycemic events were reported. Plasma glucose levels were stable or decreasing on 31 of the 32 dosing days starting at

Table I: Clinical studies of HIM2 (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Healthy volunteers	Randomized, Open, Crossover	HIM2, 0.125 mg/kg po HIM2, 0.5 mg/kg po HIM2, 0.75 mg/kg po Insulin lispro, 0.1 U/kg/d sc x 2 d	6	HIM2 monoconjugate inhibited glucose production and augmented glucose disposal in healthy subjects. It might be effective in patients with diabetes mellitus	36
Type 1 diabetes	Open	HIM2, 0.6 mg/kg po bid x 2 HIM2, 0.8 mg/kg po bid x 2 HIM2, 1 mg/kg po bid x 2	16	HIM2 was well tolerated and demonstrated glucose-stabilizing effects in patients with type 1 diabetes	40
Type 2 diabetes	Randomized, Crossover	HIM2, 0.375 mg/kg HIM2, 0.5 mg/kg HIM2, 1 mg/kg Insulin, 8 IU sc Placebo	18	HIM2 was safe and well tolerated and as effective as insulin in controlling postprandial glycemia in patients with type 2 diabetes	41

20 min after the first HIM2 dose. In some patients, after reductions or stabilization of plasma glucose levels for 30 min to 2 h, increases were observed. However, during the postdose period, most of the patients (68.8%) had plasma glucose levels that were < 150% of predose values. Plasma glucose AUC values were found to be inversely correlated with plasma insulin AUCs. HIM2 prevented the expected rise in plasma glucose in insulin-deprived patients with type 1 diabetes (37).

The efficacy and tolerability of HIM2 (0.15, 0.3 and 0.6 mg/kg p.o.) were examined in a randomized, double-blind, double-dummy, placebo-controlled, crossover study in 8 fasting and insulin-free patients with type 1 diabetes; some patients also received recombinant human insulin (4 IU s.c.). HIM2 was well tolerated, with no hypoglycemic events reported. Dose-related increases in mean plasma insulin levels were observed in patients receiving HIM2, with peak occurring by 15 min postdosing; levels returned to baseline within 120 min. A marked increase in insulin levels was observed at 0.6 mg/kg in 88% of the patients. In patients administered HIM2 alone, the rise in plasma glucose was prevented or attenuated, with effects lasting for up to 120 min (38).

A dose-escalating study conducted in 15 patients with type 1 diabetes also receiving continuous s.c. insulin infusion therapy evaluated the effects of HIM2 (0.5 and 1 mg/kg p.o.) on blood glucose levels in fasting (8 h) and fed (30 min after a standardized meal) states. HIM2 was well tolerated, with no significant adverse events observed. One patient experienced symptomatic hypoglycemia after receiving HIM2 in both the fasted and fed state. Hypoglycemic responses were observed in both fed and fasted patients receiving both doses of HIM2, although responses were more consistent with 1 mg/kg (39).

The efficacy of multiple-dose premeal HIM2 (0.25 mg/kg p.o., followed by an additional dose at 120 min postmeal) on postprandial blood glucose concentrations was compared to insulin lispro (0.1 U/kg) in a randomized study in 31 patients with type 1 diabetes (total daily insulin requirement = 1 U/kg). Doses of the insulins were titrated according to postprandial blood glucose concentrations at 240 min (100 mg/dl: decrease dose by 20%; 100-200 mg/dl: same dose; 200 mg/dl: increase dose by 50% for HIM2 or 20% for insulin lispro). A similar safety profile was obtained for both agents. However, preliminary results suggested that the incidence of gastrointestinal adverse events was higher in the group receiving HIM2. Both agents were shown to maintain 2-h postprandial blood glucose concentrations on day 3 at pretreatment levels (80-250 mg/dl) in a comparable manner (40).

The efficacy and safety of HIM2 were demonstrated in a randomized, single-blind, placebo-controlled, crossover, dose-escalating study in 18 patients with type 2 diabetes. Patients were administered oral placebo, oral HIM2 (0.375, 0.5 or 1 mg/kg) or s.c. regular insulin (Humulin; 8 U) 30 min before a standardized meal. Lower mean glucose $AUC_{0\text{-}240}$ values were obtained after administration of 0.5 (1097 mg·h/dl *vs.* 1196.9 mg·h/dl) and 1 mg/kg (801.1 mg·h/dl *vs.* 992.1 mg·h/dl) HIM2 as compared to placebo, with significant differences observed at the higher dose. Insulin $AUC_{0\text{-}240}$ values were 169.9, 193.1 and 230.8 μU·h/ml for the respective HIM2 doses as compared to 165.8, 196.1 and 169.3 μU/h/ml, respectively, for placebo. Similar mean glucose $AUC_{0\text{-}240}$ values were obtained for 0.5 and 1 mg/kg HIM2 and s.c. insulin, and pooled HIM2/s.c. insulin ratios of 2-h postprandial glucose concentrations and glucose $AUC_{0\text{-}240}$ indicated equal glucodynamic effects. However, peripheral insulin concentrations in patients receiving HIM2 were lower than those receiving s.c. insulin (mean insulin $AUC_{0\text{-}240}$ = 193.1 μU·h/ml [0.5 mg/kg] *vs.* 233.6 μU·h/ml and 230.8 μU·h/ml [1 mg/kg] *vs.* 270.3 μU·h/ml). Results suggest that HIM2 may manage postprandial glycemia without inducing hyperinsulinemia in patients with type 2 diabetes (41).

HIM2 continues to undergo phase II development for the treatment of type 1 and 2 diabetes (42, 43).

Source

Nobex Corporation (US).

References

1. Prous Science Drug R&D Backgrounders: *Diabetes mellitus (online publication)*. Updated January 9, 2004.

2. Saudek, C.D. *Progress and promise of diabetes research.* JAMA - J Am Med Assoc 2002, 287: 2582-4

3. Seidell, J.C. *Obesity, insulin resistance and diabetes - a worldwide epidemic.* Br J Nutr 2000, 83(6, Suppl.1): S5-8.

4. *The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group.* New Engl J Med 1993, 329: 977-86.

5. *Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.* Diabetes Care 1997, 20: 1183-97.

6. Mohan, V., Vijayaprabha, R., Rema, M. *Vascular complications in long-term south Indian NIDDM of over 25 years' duration.* Diabetes Res Clin Pract 1996, 31: 133-40.

7. Beghi, E., Monticelli, M.L. *Diabetic polyneuropathy in the elderly. Prevalence and risk factors in two geographic areas of Italy. Italian General Practitioner Study Group (IGPSG).* Acta Neurol Scand 1997, 96: 223-8.

8. Bavenholm, P., de Faire, U., Landou, C., Efendic, S., Nilsson, J., Wiman, B., Hamsten, A. *Progression of coronary artery disease in young male post-infarction patients is linked to disturbances of carbohydrate and lipoprotein metabolism and to impaired fibrinolytic function.* Eur Heart J 1998, 19: 402-10.

9. *Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group.* Lancet 1998, 352: 837-53.

10. Ohkubo, Y., Kishikawa, H., Araki, E., Miyata, T., Isami, S., Motoyoshi, S., Kojima, Y., Furuyoshi, N., Shichiri, M. *Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: A randomized prospective 6-year study.* Diabetes Res Clin Pract 1995, 28: 103-17.

11. Sorbera, L.A., Leeson, P.A. *Insulin glulisine.* Drugs Fut 2003, 28: 1055-8.

12. McIntyre, J.A., Bayés, M. *Insulin detemir.* Drugs Fut 2003, 28: 747-53.

13. Henry, R.R., Gumbiner, B., Ditzler, T., Wallace, P., Lyon, R., Glauber, H.S. *Intensive conventional insulin therapy for type II diabetes. Metabolic effects during a 6-mo outpatient trial.* Diabetes Care 1993, 16: 21-31.

14. Feskens, E.J., Kromhout, D. *Hyperinsulinemia, risk factors, and coronary heart disease. The Zutphen Elderly Study.* Arterioscler Thromb 1994, 14: 1641-7.

15. Schrezenmeir, J. *Hyperinsulinemia, hyperproinsulinemia and insulin resistance in the metabolic syndrome.* Experientia 1996, 52: 426-32.

16. Haenni, A., Reneland, R., Lind, L., Lithell, H. *Serum aldosterone changes during hyperinsulinemia are correlated to body mass index and insulin sensitivity in patients with essential hypertension.* J Hypertens 2001, 19: 107-12.

17. Hoffman, A., Ziv, E. *Pharmacokinetic considerations of new insulin formulations and routes of administration.* Clin Pharmacokinet 1997, 33: 285-301.

18. Price, C.H., Cherrington, A.D., Dandona, P., Clement, S., Still, J.G. *The oral delivery of insulin: Significant challenge, incomparable benefits.* Annu Meet Am Assoc Adv Sci (Feb 15-20, San Francisco) 2001, A37.

19. Still, J.G. *Development of oral insulin: Progress and current status.* Diabetes/Metab Res Rev 2002, 18(Suppl. 1): S29-37.

20. Radha Krishnan, B., Ramaswamy, M., Rajagopalan, J.S., Xu, Y., Burnham, J., Allaudeen, H.S. *Oral delivery of insulin: Single selective modificaction at B29-Lys with amphiphilic oligomer.* Annu Meet Am Assoc Pharm Sci (AAPS) (Nov 14-18, New Orleans) 1999, Abst.

21. Allaudeen, H., Ramaswamy, M., Radhakrishnan, B., Rajagopalan, J., Rajotte, RV. *Orally active insulin: A single insulin conjugate selected for future studies.* Diabetes 1999, 48(Suppl. 1): Abst 0453.

22. Radha Krishnan, B., Rajagopalan, J.S., Burnham, J. *Stability and physical characteristics of orally active amphiphilic human insulin analog, methoxy(polyethylene glycol)hexanoyl human insulin (HIM2).* Proc Int Symp Control Release Bioact Mater 2000, 27: 1038-9.

23. Sangal, D., Puskas, M., Radha Krishnan, B. *Evaluation of molecular weight distribution of poly-dispersed insulin oligomer conjugate (HIM2 poly-dispersed).* 226th ACS Natl Meet (Sept 7-11, New York) 2003, Abst MEDI 322.

24. Miller, M.A., Malkar, N.B., Odenbaugh, A.L. et al. *Effect of amphiphilic oligomers on oral insulin conjugates.* 225th ACS Natl Meet (March 23-27, New Orleans) 2003, Abst MEDI 267.

25. Malkar, N., Juska, D., Fields, G.B., Ekwuribe, N.N., James, K.D. *Effect of amphiphilic oligomers on oral insulin conjugates. Part 2: Conformational change of conjugates.* 225th ACS Natl Meet (March 23-27, New Orleans) 2003, Abst MEDI 268.

26. James, K.D., Willie, K., Malkar, N.B., Severynse-Stevens, D., Ekwuribe, N.N. *Effect of amphiphilic oligomers on oral insulin conjugates. Part 3: Solubility and protease stability.* 225th ACS Natl Meet (March 23-27, New Orleans) 2003, Abst MEDI 269.

27. Surguladze, D.N., Anderson, W.R., Filbey, J.A., Higgins, A.J. *Insulinization of the liver in normal mice by oral delivery of HIM2, a novel modified insulin.* Diabetes 2002, 51(Suppl. 2): Abst 542-P.

28. DiCostanzo, C.A., Moor, M.C., Converse, M., Scott, M., Farmer, B., Everett, C., Wilding, L., Still, J.G., Higgins, A., Cherrington, A. *Simulated first-phase insulin release is associated with prolonged improvement in post-prandial glycemia.* Diabetes 2002, 51(Suppl. 2): Abst 1284-P.

29. Boyer, J.L., Smalley, M.B., Lee, N.R., Conliffe, P.R. *Optimization of an Origen® assay for an oral insulin conjugate and comparison with a validated radioimmunoassay.* Annu Meet Am Assoc Pharm Sci (AAPS) (Oct 29-Nov 2, Indianapolis) 2000, Abst 3153.

30. Ramaswamy, M., Anderson, W.R., Still, G.J. *Dose-dependent oral absorption of insulin conjugate in normal beagles.* Annu Meet Am Assoc Pharm Sci (AAPS) (Oct 29-Nov 2, Indianapolis) 2000, Abst 3148.

31. Anderson, W.R., Segarra, I., Higgins, A.J. *HIM2, a novel modified insulin, has improved systemic pharmacokinetics in normal dogs, compared to unmodified insulin.* Diabetes 2002, 51(Suppl. 2): Abst 514-P.

32. Perry, B., Higgins, A., Holmes, N., Sinko, P.J. *Effects of gastric pH and emptying on effectiveness of liquid HIM2 insulin formulations in conscious IVAP beagle dogs.* Annu Meet Am Assoc Pharm Sci (AAPS) (Nov 10-14, Toronto) 2002, Abst T2190.

33. Soltero, R.A., Rehlaender, B., Hickey, A.J. *Evaluation of blood glucose lowering with bile salt formulations of a conjugated insulin.* Annu Meet Am Assoc Pharm Sci (AAPS) (Nov 10-14, Toronto) 2002, Abst T2164.

34. Clement, S., Still, J.G., Kosutic, G. *The effects of timing of a standardized meal on absorption of a single oral dose of hexyl-insulin monoconjugate (HIM2) in patients with type 1 diabetes.* Diabetes 2002, 51(Suppl. 2): Abst 198-OR.

35. Meyerhoff, C., Stern, W., Beer, B., Ramasway, M., Allaudeen, H. *Orally active insulin: Proof of concept study in normal volunteers.* Diabetes 1999, 48(Suppl. 1): Abst 0452.

36. Wajcberg, E., Myiazaki, Y., Triplitt, C., Cersosimo, E., DeFronzo, R.A. *Dose response effect of a single dose of orally administered hexyl-insulin monoconjugate (HIM2) in healthy nondiabetic subjects.* 18th Int Diabetes Fed Congr (Aug 24-29, Paris) 2003, Abst 805.

37. Clement, S., Still, J.G., Kosutic, G., McAllister, R.G. *Oral insulin product hexyl-insulin monoconjugate 2 (HIM2) in type 1 diabetes mellitus: The glucose stabilization effects of HIM2.* Diabetes Technol Ther 2002, 4: 459-66.

38. Still, J.G., McAllister, R.G. *Effects of orally active modified insulin in type I diabetic patients.* Clin Pharmacol Ther 2001, 69(2): Abst OIII-B-3.

39. Dandona, P., Clement, S., Still, J.G., Kosutic, G. *Effect of an oral modified insulin on blood glucose levels in fasting and fed type 1 diabetic patients receiving a "basal" regimen of injected insulin.* Diabetes 2001, 50(Suppl. 2): Abst 177-OR.

40. Clement, S., Kipnes, M., Steinberg, H., Fineberg, S.E., Jovanovic, L., Rikalo, N., Marks, J., Gibson, D., Still, J.G., Kosutic, G. *Effects of multiple doses of orally administered hexyl insulin M2 (HIM2) on postprandial blood glucose (PPG) concentrations in type 1 diabetic (T1) patients.* 62nd Annu Meet Sci Sess Am Diabetes Assoc (June 14-18, San Francisco) 2002, Abst 11-LB.

41. Kipnes, M., Dandona, P., Tripathy, D., Still, J.G., Kosutic, G. *Control of postprandial plasma glucose by an oral insulin product (HIM2) in patients with type 2 diabetes.* Diabetes Care 2003, 26: 421-6.

42. *Product pipeline.* Nobex Web Site December 16, 2003.

43. *Nobex to reacquire modified insulin molecule rights from GlaxoSmithKline.* DailyDrugNews.com (Daily Essentials) November 18, 2003.

Additional References

Clement, S., Still, J.G., Kosutic, G., Mc Allister, R. *A dose-escalation study of the effects of two sequential doses of oral modified insulin on blood glucose concentrations in patients with type 1 diabetes mellitus.* Diabetes 2001, 50(Suppl. 2): Abst 435-P.

Radha Krishnan, B., Puskas, M., Sangal, D., Xu, Y., Rajagopalan, J.S. *Evaluation of molecular weight distribution of orally active insulin conjugate HIM2.* Annu Meet Am Assoc Pharm Sci (AAPS) (Oct 29-Nov 2, Indianapolis) 2000, Abst 3299.

Kipnes, M., Dandona, P., Still, J.G., Kosutic, G. *The effects of an oral modified insulin on postprandial blood glucose levels in patients with type 2 diabetes.* 61st Annu Meet Sci Sess Am Diabetes Assoc (June 22-26, Philadelphia) 2001, Abst 5-LB.