Hepatic-Directed Vesicles-Insulin: Evaluation of a Novel Oral and Subcutaneous Insulin Delivery System in Animal Models of Diabetes

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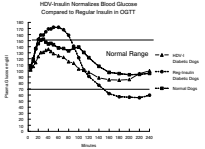
Abstract

A novel investigational liposomal (<150 nm in diameter) insulin delivery system, hepatic-directed vesicles insulin (HDV-I), that contains 1% encapsulated insulin and a specific proprietary hepatocyte targeting molecule (HTM) in its bilipid layer, has been developed. The HTM selectively targets the delivery of the encapsulated insulin to the hepatocytes in the liver in a manner similar to normal insulin physiology, in contrast to conventional SC insulin or untargeted insulin that readily distributes to peripheral tissues, fat and muscle. HDV-I is formulated for oral gel capsule and SC administration in types 1 and 2 diabetics. The results of early evaluation studies in animal models have revealed that: (1) in streptozotocin/alloxan insulin deficient dogs, administration of regular insulin (RI) at varying doses via the jugular vein resulted in conversion of glucose output to uptake at 6.25 mU/kg/min. In contrast, infusion of HDV-I through the same route resulted in conversion of hepatic glucose output to uptake at all doses tested from 0.4 to 0.025 mU/kg/min. (2) in streptozotocin/alloxan insulin deficient rats given oral glucose load containing ^{14}C-glucose as a tracer, HDV-I markedly decreased peripheral blood glucose by ~35% and increased ^{14}C-glucose uptake in the liver by 250%. In contrast, RI and saline had no effect on either. (3) In pancreatectomized dogs during an OGTT, HDV-I had a more pronounced effect than RI in reducing the PPG excursions after administration of the glucose meal and it prevented the secondary hypoglycemia that is believed to be due to the effect of RI on peripheral tissues (see Figure).



In conclusion, results of these animal studies, show that HDV-I can stimulate hepatic activity at a dose that is <1% of the dose of RI required for liver stimulation; suggest that the enhanced antihyperglycemic effect of HDV-I is due to hepatic glucose uptake; and show that HDV-I was superior to RI in reducing postprandial glycemia and was not associated with secondary hypoglycemia. HDV-I holds significant promise in the treatment of diabetes.

Background

The importance of intensive glucose management in the prevention of the microvascular and macrovascular complications in type 1 and type 2 diabetes is well established.[1-4] Type 1 diabetes patients require daily insulin or intensive insulin therapy regimens involving three or more daily injections. In type 2 diabetes, most patients will ultimately require the addition of insulin since oral antidiabetic drugs become insufficient as the disease progresses/insulin production declines.[5-7] These requirements for insulin places a heavy burden of compliance on patients and has prompted interest in developing alternative routes of insulin delivery,[8,9] Of the non-invasive alternative routes that are being developed, hepatic-directed vesicles insulin (HDV-I) more closely mimics the normal physiological delivery of insulin and represents a significant advance. HDV-I, formulated for subcutaneous (SC) and oral gelcap administration is a novel investigational liposomal (<150 nm in diameter) insulin delivery system (Diasome Pharmaceuticals, Conshohocken, PA) that contains 1% encapsulated insulin in liposomes with a specific proprietary hepatic targeting molecule (HTM) in its bilipid layer (oral form) plus 99% untargeted insulin (SC form). When administered, the HTM selectively targets the delivery of the encapsulated insulin to the hepatocytes in the liver, in contrast to conventional SC insulin or untargeted insulin that readily distributes to peripheral tissues (fat and muscle) before reaching the liver. The objectives of these early studies were to evaluate the effect of HDV-I on hepatic glucose uptake and peripheral blood glucose in various animal models of diabetes.

Objectives

Study 081095b: To compare the bio-potency of HDV-I to that of regular insulin on hepatic glucose retention of portally infused glucose in intact versus streptozotocin/alloxan insulin deficient beagle dogs. ***Study 020990:*** To investigate the effect of HDV-I on hepatic uptake of oral ^{14}C-glucose and peripheral blood glucose in insulin-deficient rats. ***Study 071598:*** To compare the efficacy of HDV-I to regular insulin (RI) on the oral glucose tolerance test (OGTT) in pancreatectomized dogs.

Materials & Methods

Animal Preparation & Experimental Procedure

Study 081095b: Normal (intact) adult beagle dogs and streptozotocin 30 mg/kg / alloxan 50 mg/kg induced diabetic beagle dogs[10] weighing 8 – 10 kg were used for the experiments. After a 24 hour fast, all dogs were anesthetized with Surital and maintained with Metaphane and oxygen. Through a midline abdominal incision, the hepatic artery was ligated so that the hepatic portal vein (HPV) was the only source of hepatic blood flow, and catheters were placed in the hepatic and portal (via the splenic vein) veins for simultaneous sampling of glucose and insulin levels. Following a baseline period, glucose 10% w/v was infused via a mesenteric vein into all animals at 0.5 g/kg/hr followed by infusion of insulin preparations (HDV-I and regular insulin) at rates of 6.25 to 0.025 mUnits/kg/hr via the external jugular or mesenteric vein in diabetic animals while saline infusion replaced insulin in the intact animals. Portal blood flow (HBF) was measured by indocyanine green dye dilution method; insulin by immunoreactive method; and glucose balance was calculated across the liver with positive and negative balance values indicating retention and output of glucose by the liver, respectively.

Study 020990: Streptozotocin (20 mg/kg IP) induced insulin-deficient female Sprague-Dawley rats (n=17) weighing 250 g were divided into 3 treatment groups: Group I (n=5) - saline 0.2 ml per rat; Group 2 (n=6) - HDV-I 0.32 U/rat in 0.2 ml volume; and Group 3 (n=6) - regular insulin (RI) 0.32 U/rat in 0.2 ml volume. Immediately after dosing with the test materials, the rats were given glucose (50% in water) IP at a dose of 1.5 g/kg spiked with ^{14}C-glucose (403,610 cpm/g glucose), simulating an oral or portal dose. At 2 hours following the injection of saline or insulin, the rats were sacrificed and a central venous blood sample was obtained for glucose measurement with a Beckman Blood Glucose Analyzer and the liver removed for counting of radiolabeled glucose in a liquid scintillation counter.

Study 071598: Surgically pancreatectomized adult mongrel dogs weighing 20 - 30 kg were stabilized on NPH insulin and a booster dose of RI with the main meal to maintain morning FBG levels of 100 – 200 mg/dl. The dogs were trained to be in Pavlov pouches during the experiments. Oral glucose tolerance test (OGTT) was performed by substituting oral glucose (1.5 g/kg as 50% glucose in water) for the morning snack and RI or HDV-I dose were administered SC 30 min and 15 min prior to the oral glucose load in Experiments 1 and 2, respectively. At least a 1 day interval was observed between the administration of the 2 drugs in a single dog and all individualized insulin doses were held constant for a given dog. Venous blood samples were taken following glucose administration according to a prespecified schedule from 5 to 240 min. Glucose was measured with an automated Beckman Blood Glucose Analyzer. Euglycemia was defined as blood glucose of 70 – 140 mg/dl, hyperglycemia as >140 mg/dl, and hypoglycemia as <70 mg/dl.

Results

In intact (normal) dogs, portal vein infusion of glucose stimulated a conversion from hepatic glucose output to hepatic glucose uptake (Figure 1A) with elevated portal vein insulin levels (Figure 1B). In contrast, in the insulin-deficient dogs, with portal vein infusion of glucose, the animals remained in hepatic glucose output (Figure 1A) with no increase in portal vein insulin levels (Figure 1B).

Results

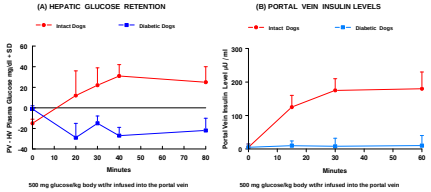


Figure 1A & 1B. Hepatic Glucose Retention in Intact and Diabetic Dogs (1A) and Portal Vein Insulin Levels (1B) Following Portal Vein Infusion of Glucose 0.5 g/kg/hr Over 80 min. HV = Hepatic vein; PV = Portal vein.

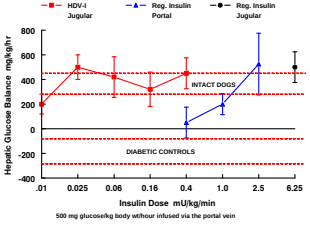


Figure 2. Hepatic Glucose Balance and Insulin Dose-Response in Intact and Diabetic Dogs (n = 4 or 6 each). The areas between the parallel lines indicate the range of hepatic glucose balance for intact and diabetic dogs in the absence of exogenous glucose ±SEM.

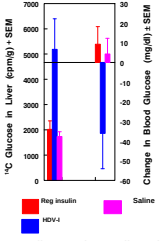


Figure 3. Figure 3: Effect of HDV-Insulin, Regular Insulin and Saline on Blood Glucose and Hepatic ^{14}C-Glucose Uptake in Insulin-Deficient Rats.

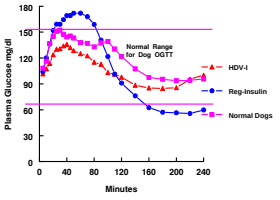


Figure 4. Effect of HDV-Insulin Dosed 30 and 15 min (Combined Data) Before Glucose Load on the OGTT in Insulin-Deficient (Pancreatectomized) Dogs (n = 6). Data for Curve of normal dogs was obtained before pancreatectomy.

Figure 2 Comments. RI infused via the external jugular vein into glucose-loaded diabetic dogs converted hepatic glucose output to hepatic glucose uptake at infusion rate of 6.25 mU insulin/kg/min but not at 2.5 mU insulin/kg/min. In contrast, infusion of HDV-I into the external jugular vein of glucose-loaded diabetic dogs was followed by conversion of hepatic glucose output to hepatic glucose uptake within 20 min and at all the much lower doses of 0.025, 0.06, 0.16 and 0.4 mU insulin/kg/min, including a dose <1% of the RI dose that stimulated the liver.

Figure 3 Comments. HDV-I administration was followed by statistically significant accumulation of ^{14}C-glucose in insulin-deficient rat livers (5184 ± 2973 cpm/g) that was 256% of the amount after the same dose of RI (2023 ± 810 cpm/g; p = 0.0308) and 299% of that after saline (1734 ± 461 cpm/g; p = 0.0314) treatment. Two hours following the injection of HDV-I, there was a reduction in the mean blood glucose value by -193.2 ± 222 mg/dl (or 36.1%) that was statistically significantly different (p = 0.0427) from the mean change in blood glucose following the administration of the same dose of RI. Between HDV-I and saline treatments, the mean change in blood glucose was lower for HDV-I and approached but did not achieve statistical significance (p = 0.0738). No significant differences between the mean change in blood glucose values following RI versus saline.

Figure 4 Comments. HDV-I and RI were equally effective in controlling glycemia with a rapid onset of effect when administered either 30 min or 15 min prior to an oral glucose load in pancreatectomized dogs. SC HDV-I treatment was effective in controlling blood glucose levels in pancreatectomized dogs following an oral glucose load, preventing both hyperglycemia and secondary hypoglycemia. In contrast, the same dose of SC RI treatment failed to control the hyperglycemia following an oral glucose load in pancreatectomized dogs and was associated with secondary hypoglycemia.

Conclusions

- HDV-I is superior to RI in controlling postprandial glycemia and was not associated with secondary hypoglycemia.
- The results suggest that the antihyperglycemic effect of HDV-I is due to hepatic glucose uptake.
- HDV-I can stimulate hepatic activity at a dose that is <1% of the dose of RI required for liver stimulation.
- HDV-I holds significant promise in the treatment of diabetes.

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