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REVIEW ARTICLE

Hepatic-Directed Vesicle Insulin: A Review of Formulation Development and Preclinical Evaluation

W. Blair Geho, M.D., Ph.D.,[1,2] Hans C. Geho, M.D., J.D.,[3] John R. Lau,[1] and Theophilus J. Gana, M.D., Ph.D.[4]

Abstract

Hepatic-directed vesicle insulin (HDV-I), a novel investigational vesicle (<150 nm diameter) insulin delivery system that carries insulin and a specific hepatocyte-targeting molecule (HTM) in its phospholipid bilayer and has the ability to mimic a portal vein insulin infusion remotely [subcutaneous (SC) HDV-I] and noninvasively (oral HDV-I), has been developed. This review summarizes formulation development, subsequent refinements, and results of preclinical evaluation studies, including biodistribution, mechanistic, and toxicology studies of predominantly SC HDV-I, in various animal models. Studies conducted to date have confirmed the hepatocyte specificity of HDV and HDV-I and revealed that HDV-I can stimulate the conversion of hepatic glucose output to uptake at a dose that is <1% of the dose of regular insulin (RI) required for liver stimulation; suggest that the enhanced antihyperglycemic effect of HDV-I is due to hepatic glucose uptake; and in pancreatectomized dogs during an oral glucose tolerance test, HDV-I normalized blood glucose curves when compared to control curves in intact dogs and prevented secondary hypoglycemia in contrast to the same dose of RI. A 28-day SC HDV toxicity study in rats revealed no clinical, clinical laboratory, or histopathological findings, and the battery of three genetic toxicology studies was negative. Results support the hypothesis that HDV-I works by stimulating hepatic glucose uptake and/or glycogen storage in insulin-deficient animals. The ability to target the delivery of HDV-I to the liver reestablishes the liver as a major metabolic modulator of glucose metabolism. The future of HDV-I depends on the results of ongoing development and longer term clinical trials.

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Introduction

Small phospholipid vesicles[1] have the ability to carry substances and, because "address labels" or specific targeting ligands can be attached to their phospholipid bilayer,[2] vesicle technology has been exploited for the targeted delivery of vaccines, cancer drugs, antimicrobials, insulin, and so on[3,4] to the specified cells within the human body where it is required.

Hepatic-directed vesicle insulin (HDV-I) is a novel investigational (<150 nm diameter) insulin delivery

Author Affiliations: [1]SDG, Inc., Cleveland, Ohio; [2]Diasome Pharmaceuticals, Inc., Conshohocken, Pennsylvania; [3]Department of Geriatrics, MetroHealth Medical Center, Cleveland, Ohio; and [4]Biopharmatech Consulting, Inc., Leesburg, Virginia

Abbreviations: (AUC) area under the concentration–time curve, (DGDG) digalactosyl diglyceride, (HDV) hepatic-directed vesicle, (HDV-I) hepatic-directed vesicle insulin, (HTM) hepatocyte-targeting molecule, (OGTT) oral glucose tolerance test, (PE) phosphatidylethanolamine, (PPG) postprandial plasma glucose, (RI) regular insulin, (SC) subcutaneous

Keywords: diabetic animals, formulation, hepatic-directed vesicle insulin, hepatic glucose retention, intact animals, vesicle insulin delivery system

Corresponding Author: W. Blair Geho, M.D., Ph.D., SDG, Inc., 10265 Carnegie Avenue, Cleveland, OH 44106; email address *bgeho@diasome.com*

system developed by Diasome Pharmaceuticals, Inc.[5–9] It is formulated for subcutaneous (SC) and oral gel cap administration. The SC HDV-I formulation contains 1% bound insulin and 99% untargeted free insulin. However, the oral formulation has no free insulin and all the insulin is bound to HDV. The vesicles contain a specific proprietary hepatocyte-targeting molecule (HTM), biotin-phosphatidylethanolamine (biotin-PE), in their phospholipid bilayer (SC and oral formulations). Following administration, HDV enhances the absorption of the insulin it carries (oral HDV-I) by preventing its degradation by the proteolytic enzymes in the upper gastrointestinal tract, whereby the HTM selectively targets delivery of the insulin to the hepatocytes in a manner similar to normal physiological insulin delivery. This review summarizes the history of HDV-I formulation development and subsequent refinements, along with the biodistribution, mechanistic, and toxicology studies conducted to evaluate SC HDV-I in various diabetic and nondiabetic animal models. Oral HDV-I is briefly mentioned as well.

Rationale: Why Target Insulin Delivery to Hepatocytes?

The rationale for selectively targeting the delivery of insulin to the liver as compared to conventional SC administration into the systemic (peripheral) circulation has been well discussed previously.[4,10,11] Briefly, in patients with diabetes, the portal-systemic insulin concentration gradient that ensures that the liver is exposed to concentrations of insulin that are two to three times higher than the peripheral circulation[12–14] is reversed due to lack of insulin (type 1 diabetes) or insufficient production/decreased sensitivity of the liver to insulin (type 2 diabetes). Therefore, the unphysiological conventional SC injection of insulin into the peripheral circulation results in additional peripheral hyperinsulinemia,[15–17] which is known to be associated with atherosclerosis,[18] cancer,[19] hypoglycemia, and other adverse metabolic effects.[20–22] In contrast, some reports have indicated that intraportal insulin infusion or adequate hepatic insulinization required lower doses of insulin and was associated with more rapid and significant lowering of plasma glucose and hemoglobin A1c levels, normalization of plasma levels of three carbon precursors such as lactate, pyruvate, and alanine, and the hormones cortisol, growth hormone, and glucagon. These findings were accompanied by lower fasting plasma hyperinsulinemia compared to conventional SC insulin infusion in patients with type 1 diabetes,[16] as well as in pancreatectomized dogs.[23,24] HDV-I is being developed to provide the physician with the ability to mimic a portal vein insulin infusion both remotely (SC HDV-I) and noninvasively (oral HDV-I).

Liposome Manufacture and Formulation Development

Subcutaneous HDV–Insulin

The original HDV was designed as a nano carrier for insulin. Detailed descriptions of the HDV compositions can be found in the relevant U.S. patents.[25–28] The first version of HDV was manufactured using sonication methodology and was composed of distearoyl lecithin—the structural phospholipids; dicetyl phosphate—provided a net negative charge to the carrier; cholesterol—gave added bilayer membrane stability; and digalactosyl diglyceride (DGDG)—conferred hepatocyte specificity by virtue of affinity for either the asialoglycoprotein (galactose) or Ashwell receptor[29] or, in subsequent HDV versions, the hepatobiliary or biotin receptor. The latter components were sonicated together with regular pork insulin (Iletin regular pork, Eli Lilly & Company, Indianapolis, IN, or Actrapid Novo regular pork insulin, Novo Nordisk Inc., Princeton, NJ). Any unbound insulin (free insulin) was separated from the vesicles by Sephadex G-100 chromatography (**Figure 1**). ^{125}I-Insulin (Amersham, Buckinghamshire, UK) was added to the insulin solutions prior to sonication in order to quantitate the insulin content of the HDV. The biological activity of HDV-I was confirmed by dissolving the vesicles in chloroform and methanol, extracting the insulin, and demonstrating the expected hypoglycemic activity of the insulin in rats. The physical structure of HDV was determined and monitored by negative stain transmission electron microscopy. The HDV thus produced were unilamellar vesicles of 25 to 125 nm in diameter with incorporation

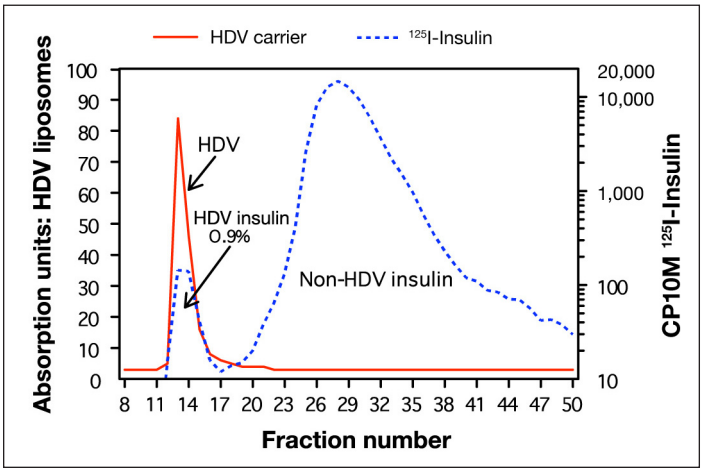

Figure 1. Chromatogram showing separation of [^{125}I]insulin from the HDV–insulin mixture. HDV, hepatic-directed vesicle.

of insulin, giving a final HDV-I concentration of up to 10 IU/ml.

The HDV has subsequently undergone a series of formulation refinements that have steadily improved its function as a hepatocyte delivery vehicle. A second version of HDV incorporated biliverdin and disofenin,[26–28] which targets the hepatobiliary receptor, as HTM instead of DGDG because the uptake of DGDG could potentially be competitively inhibited at the Ashwell receptor by other glycoproteins bearing terminal galactose, a blocking phenomenon that was first described by Morell *et al.*[30] This disofenin-containing HDV and the subsequent third version of HDV were manufactured by microfluidization (using Model #M-110EH-30K, MicroFluidics, Lowell, MA) instead of sonication. When human recombinant regular insulin (Humulin® R, Eli Lilly & Company, or Novolin® R, Novo Nordisk, Inc.) became available, it replaced porcine insulin. The test formulation became 11.3 mg HDV in a 0.8-ml volume added to 10 ml commercial IU-100 Humulin R. This amount of HDV bound 10 IU or 1% of insulin, leaving 990 IU regular insulin unattached to HDV. This product (SC HDV-I) was validated in a series of animal bioassay and pharmacology studies, the results of which are presented here. HDV-I has been manufactured and scaled up under Good Manufacturing Practice conditions and used for an oral glucose tolerance study in human type 1 diabetes patients[5] and in a 28-day SC HDV repeated-dose, Food and Drug Administration-designed rat Good Laboratory Practice toxicity study.

Although the amount of chromium dosed in the previous two versions of the formulation was small (less than 10 ng/kg body weight), there was some concern about the presence of any chromium in the formulation. Therefore, the latest versions of SC and oral HDV-I use biotin-PE as the HTM, otherwise it is the same formulation as for disofenin. The biological activity of SC and oral HDV with biotin-PE as HTM has been tested in several rat bioassay studies and has been shown to be as effective as HDV with disofenin chromium; in some instances it was superior.

Oral HDV–Insulin Formulation

Hepatic-directed vesicle insulin has also been formulated for oral delivery. Insulin-binding studies with HDV have resulted in a formulation in which 1 IU regular recombinant insulin is tightly bound by 1 mg HDV. In this mixture, all insulin is bound to HDV, unlike the dosage form that has been used for SC administration, described earlier. The HDV-I in this dosage form can be formulated into a dry mix using a proprietary procedure that results in a 5-unit HDV-I size 2 capsule for oral administration. This solid oral dosage form has insulin stability at 5, 25, and 40°C for 5 months (unpublished data). With respect to stability in aqueous systems, SC HDV-I is stable for 30 days at room temperature.

Dose Equivalency of Oral HDV–Insulin to Injectable HDV–Insulin and Regular Insulin. Oral HDV-I formulation has been demonstrated in a number of clinical trials to be equivalent to injected insulin, dose for dose, in controlling postprandial hyperglycemia. In an open-label, randomized, active-controlled and placebo-controlled study to demonstrate the efficacy and safety of oral HDV-I when administered prior to an oral glucose tolerance test (OGTT) in adult patients with type 1 diabetes mellitus, results demonstrated that single doses of 0.1 and 0.2 U/kg oral HDV-I provided identical postprandial glycemic control, as indicated by incremental plasma glucose area under the concentration–time curve (AUC) results (**Figure 2A**).[31] Furthermore, the postprandial glycemic control produced by 0.1 and 0.2 U/kg oral HDV-I was similar to that produced by 0.07 U/kg SC Humulin R (the dose determined to be adequate to handle a standard diet meal containing 60 grams of carbohydrate). Oral HDV-I administration did not appear to be associated with a dose–response similar to a previous observation following SC Humulin R administration. This finding confirmed the lack of a dose–response seen in earlier diabetic dog studies with HDV-I and demonstrated dose equivalency with respect to the antihyperglycemic effect of 0.1 and 0.2 U/kg oral HDV-I versus 0.07 U/kg SC Humulin R.

In another multicenter (three sites), randomized, double-blind (SC HDV-I and SC Humulin R) and open-label (oral HDV-I) study in adult type 1 diabetes patients on basal glargine therapy (Lantus®; Sanofi-Aventis U.S. LLC, Bridgewater, NJ) over a 14-day period, we evaluated the antihyperglycemic efficacy and safety of SC and oral HDV-I formulations in comparison to SC Humulin R.[6] Patients (n = 30) were titrated to stable doses of insulin glargine twice daily plus three premeal Humulin R injections and Humulin R prior to snacks over a 14-day baseline stabilization period. Patients were then randomized to receive 0.07 U/kg SC Humulin R (n = 11), 0.07 U/kg SC HDV-I (n = 11), or 0.1 U/kg oral HDV-I (n = 8) 15 minutes before breakfast, lunch, and dinner if they had 3 consecutive days of fasting plasma glucose levels <120 mg/dl and 1-hour postprandial plasma glucose (PPG) levels <170 mg/dl. Patients measured/recorded daily fasting blood glucose before breakfast; daily 2-hour PPG following lunch and dinner; a seven-point blood glucose test on days 1, 4, 7 and 11; and

adverse/hypoglycemic events in a patient diary. Results revealed that oral HDV-I and SC HDV-I significantly reduced ($p < 0.05$), whereas SC Humulin R increased ($p = 0.087$) the overall mean daily seven-point blood glucose at end point (**Figure 2B**). Only the mean change from baseline by SC HDV-I was significantly different compared to SC Humulin R; the mean reduction by oral HDV-I approached ($p = 0.074$) but did not achieve statistical significance, probably due to the small sample size. Between treatments, the magnitude of reductions in the overall mean seven-point blood glucose values was similar except for the SC HDV-I treatment, which was significantly ($p = 0.014$) different from the mean increase observed for the SC Humulin R treatment. In addition, the 0.1-U/kg oral HDV-I treatment was associated with the same rate but lower magnitude of improvement in mean daily seven-point blood glucose levels as the same dose of SC HDV-I, as indicated by an identical negative slope of the best-curve fit.

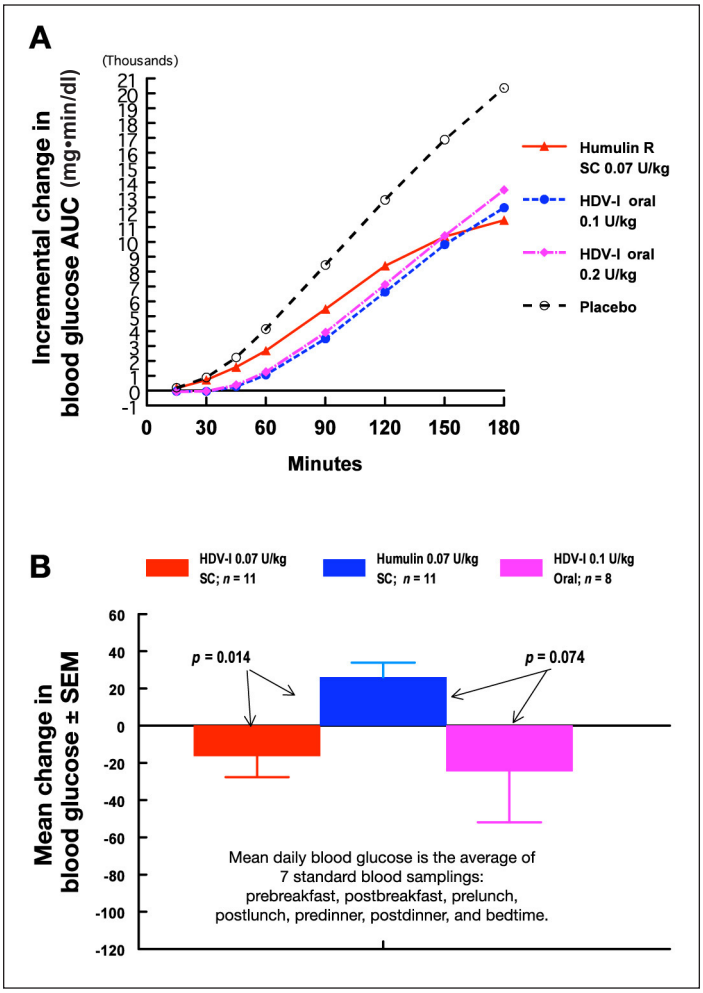

Figure 2. (A) Mean incremental plasma glucose AUC plots following the administration of single doses of 0.1 and 0.2 U/kg oral HDV–insulin versus subcutaneous 0.07 U/kg Humulin R and oral placebo ($n = 4$). Mean incremental plasma glucose AUC plots for the two doses of oral HDV–insulin were almost identical, indicating the lack of a dose–response, and were similar to the mean value obtained for subcutaneous Humulin R over the 180-minute study period. AUC, area under the concentration–time curve; HDV, hepatic-directed vesicle. **(B)** Comparison of overall mean ± SEM daily seven-point blood glucose values among SC HDV–insulin, SC Humulin R, and oral HDV–insulin treatments. Between treatments, only the mean reduction in the overall mean seven-point blood glucose value by SC HDV–insulin treatment was significantly ($p = 0.014$) different from the mean increase observed for the SC Humulin R treatment. SEM, standard error of the mean; SC, subcutaneous; HDV, hepatic-directed vesicle.

Evaluation of HDV–Insulin in Animal Studies

HDV and HDV–Insulin Biodistribution and Hepatocyte Specificity Studies in Mice and Rats

Following the production of HDV-I, biodistribution and hepatocyte-specific localization studies were conducted in mice and rats. The biodistribution of a bolus intravenous dose of HDV (labeled with [^{14}C]cholesterol and prepared with and without insulin) was studied in two groups of 16 intact mice. Results showed that injectable HDV, with and without insulin, disappeared rapidly from the blood and appeared rapidly in the liver, with approximately 80% of the radioactivity appearing in the liver within 60 minutes of dosing. Minimal amounts were found in the spleen. Results of that study were published previously by Davis and colleagues.[5] In addition, the hepatocyte specificity of the injectable HDV delivery system was demonstrated in rats by enclosing 5-nm gold particles within hepatic-targeted vesicles (with disofenin as HTM) versus nonhepatic-targeted vesicles administered intravenously and thereafter examining the liver at various time periods to determine the cellular localization of the vesicles. Using a silver enhancement technique, the gold particles were located throughout the hepatocytes, which indicated intracellular localization of the hepatic-targeted vesicles within the liver (**Figure 3A**) as compared to the location of nonhepatic-targeted vesicles in the Kupffer cells within the liver (**Figure 3B**). Results of these studies confirmed the selective distribution of injectable HDV and HDV-I to the liver and their hepatocyte specificity following administration.

Hepatic Glucose Balance Study in Dogs

The *in vivo* biopotency of the first version of HDV-I (with DGDG as the HTM and all free insulin removed by Sephadex G-100 chromatography) on hepatic glucose balance was compared to that of regular insulin in intact (normal) versus diabetic beagle dogs ($n = 6$/group; 8–10 kg). Insulin deficiency was induced by streptozotocin/alloxan in beagle dogs according to the method described

by Black and colleagues.[32] The diabetic dogs were maintained on 2–8 IU/day of ultralente insulin to ensure that fasting blood glucose was approximately 250 mg/dl and that the dogs were not ketotic and had stable body weights. Under anesthesia and following a midline abdominal incision, the hepatic artery was ligated so that the hepatic portal vein was the only source of hepatic blood flow, and catheters were placed in a portal vein (hilus) via the splenic vein for sampling the hepatic vein and external jugular vein. Glucose and indocyanine green were infused via scalp vein needles placed in mesenteric veins. Following a baseline period, 10% (w/v) glucose was infused via a mesenteric vein into all animals at 0.5 g/kg/hr followed by the infusion of insulin preparations at different rates ranging from 6.25 to 0.025 mU/kg/hr via the external jugular or mesenteric vein in diabetic animals while saline infusion replaced insulin in the intact animals. The simplified hepatic circulation (with the hepatic artery ligated) and the portal and hepatic blood glucose differences multiplied by portal blood flow provided the glucose balance across the liver with positive and negative balance values indicating hepatic glucose uptake and hepatic glucose output, respectively, using formulas published previously.[33]

In the intact dogs, an intramesenteric vein infusion of 0.5 g/kg/hr glucose increased portal vein glucose from a baseline value of 120 mg/dl to over 300 mg/dl at 80 minutes (**Figure 4A1**) with elevated portal vein insulin levels (**Figure 4A2**), stimulating a conversion from hepatic glucose output to hepatic glucose uptake. In contrast, in the diabetic dogs, an intramesenteric vein infusion of 0.5 g/kg/hr glucose increased portal vein glucose from a baseline value of approximately 250 mg/dl to over 400 mg/dl with no increase in portal vein insulin levels but the animals remained in hepatic glucose output.

Regular insulin (RI) infused intravenously into glucose-loaded diabetic dogs suppressed hepatic glucose output and stimulated hepatic glucose uptake in a dose-dependent fashion and, as expected, the dose–response relationships varied with the route of insulin administration (**Figure 5**). RI infusion via the external jugular vein into glucose-loaded diabetic dogs converted hepatic glucose output to hepatic glucose uptake only at the high infusion rate of 6.25 mU/kg/min (**Figures 4B1** and **5**) but not at 2.5 mU/kg/min, with the portal vein insulin levels elevated to values similar to those of glucose-loaded intact animals at 6.25 mU/kg/min (**Figure 4B2**). The portal vein insulin levels at 2.5 mU/kg/min RI were much lower than at 6.25 mU/kg/min. With portal vein infusion of RI in diabetic dogs, the rates of insulin administration

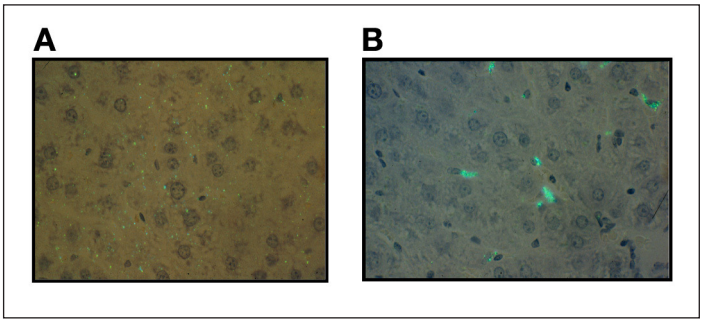

Figure 3. Light microscopic localization of hepatic-targeted vesicles (HDV with disofenin as HTM) versus nonhepatic-targeted vesicles (HDV without HTM) containing 5-nm gold particles in frozen sections of rat liver. **(A)** Uniform intracellular (hepatocyte) localization of silver-enhanced, 5-nm gold particles as small greenish dots enclosed in hepatic-targeted vesicles. **(B)** Uptake (clumping) of silver-enhanced, 5-nm gold particles by Kupffer cells as larger greenish dots enclosed in nonhepatic-targeted vesicles. HTM, hepatocyte-targeting molecule; HDV, hepatic-directed vesicle.

required to convert hepatic glucose output to net hepatic glucose uptake were lower than required with the peripheral route (**Figure 5**). Therefore, net glucose uptake was seen at the lower portal infusion rates of 2.5 and 1.0 mU/kg/min. In contrast, infusion of HDV-I into the external jugular vein when intraportal glucose was infused into diabetic dogs resulted in a conversion of hepatic glucose output to hepatic glucose uptake within 20 minutes and at the much lower doses of 0.4, 0.16, 0.06, and down to 0.025 mU/kg/min (**Figures 4C1** and **5**). No portal vein insulin was measurable by standard radioimmunoassay in dogs receiving the 0.4-mU/kg/min infusion of HDV-I (**Figure 4C2**). The conversion from hepatic glucose output to hepatic glucose uptake accomplished with these very small doses of HDV-I was not accompanied by measurable increases in portal insulin levels, as contrasted with the efficacious peripheral infusion of regular insulin. HDV-I (0.05 IU/kg) was also effective in restoring hepatic glucose uptake in insulin-deficient dogs when administered subcutaneously, but regular insulin at this dose did not. Portal insulin values were not obtained in these two treatment groups.

Portal glucose administration causes conversion of hepatic glucose output to hepatic glucose uptake in intact dogs; however, diabetic dogs remain in hepatic glucose output. Results of this glucose balance study showed that both RI and HDV-I containing only 1% bound insulin induced the conversion of hepatic glucose output to hepatic glucose uptake when infused into diabetic dogs at various doses via the external jugular vein; however, HDV-I produced its effect at the much lower doses (0.025 to 0.4 mU/kg/min) administered, including a dose that was <1% of the only dose of RI (6.25 mU/kg/min) that

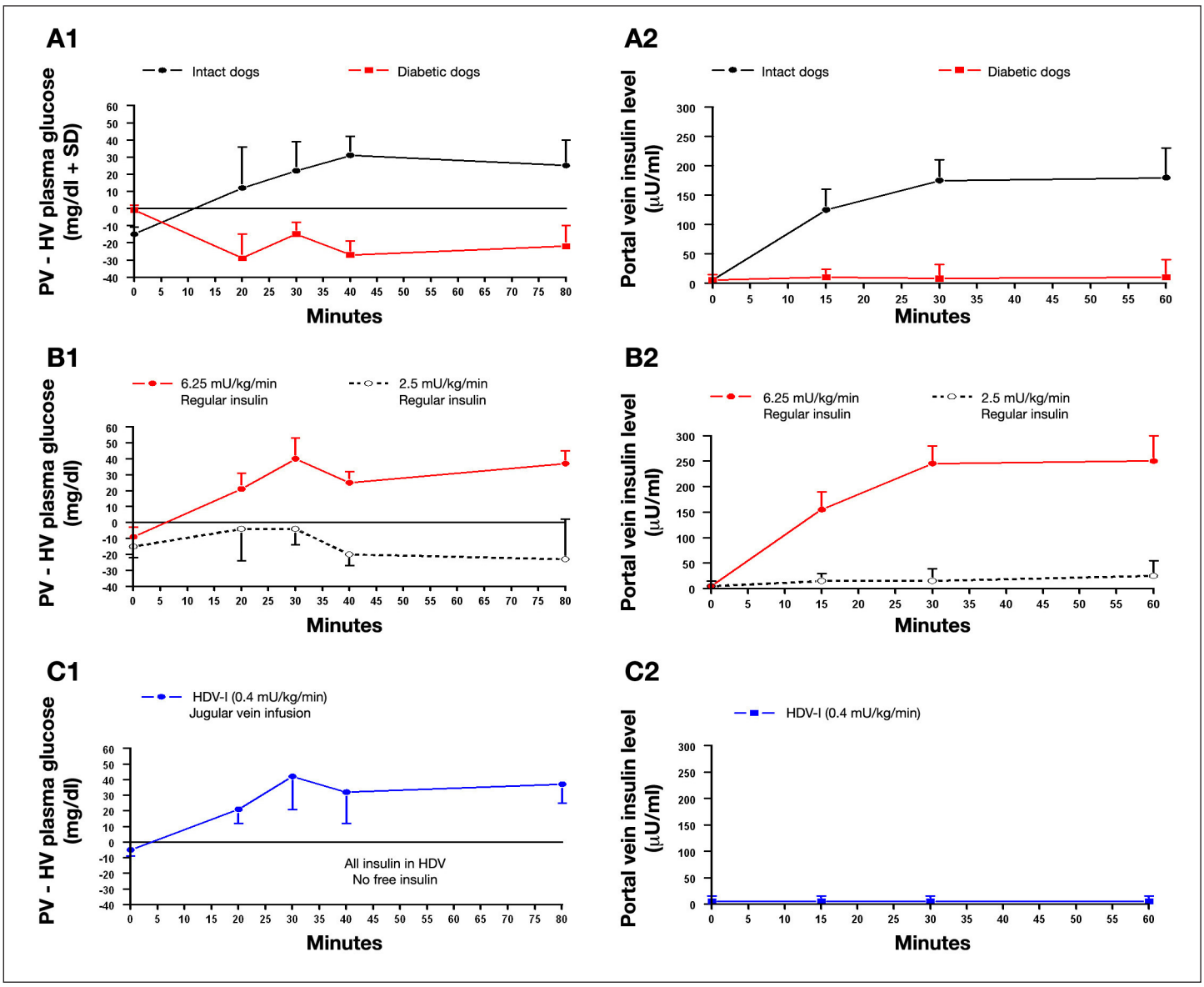

Figure 4. Hepatic glucose retention **(A1)** and portal vein insulin levels **(A2)** in intact and diabetic dogs following portal vein infusion of 0.5 g/kg/hr glucose over 80 minutes. Hepatic glucose retention in diabetic dogs **(B1)** and portal vein insulin levels in diabetic dogs **(B2)** during external jugular vein infusion of 2.5 and 6.25 mU/kg/min regular insulin and portal vein infusion of 0.5 g/kg/hr glucose over 80 minutes. Hepatic glucose retention in diabetic dogs **(C1)** and portal vein insulin levels in diabetic dogs **(C2)** during external jugular vein infusion of 0.4 mU/kg/min HDV-I and portal vein infusion of 0.5 g/kg/hr glucose over 80 minutes. HV, hepatic vein; PV, portal vein; n = 6 dogs per group; HDV, hepatic-directed vesicle.

stimulated the liver. Furthermore, no increase in effect was observed following an increase in the dose of HDV-I administered. These results indicate an efficient delivery of insulin and suggest that a direct hepatic effect of HDV-I is required to restore hepatic glucose uptake and perhaps normal glucose tolerance.

Lack of Metabolic Activity of HDV Compared to HDV–Insulin Vesicles. Experiments were also conducted to establish that empty vesicles (not entrapping insulin) have no metabolic activity. Injectable HDV (containing DGDG as the HTM) prepared in the absence of insulin, administered in an amount 10 times greater than that given with the highest HDV-I infusion (0.4 mU/kg/min), did not cause any alteration in hepatic glucose metabolism in the glucose-infused diabetic dog. At the conclusion of a 30-minute infusion of empty vesicles, the 0.4-mU/kg/dose of insulin-loaded vesicles (HDV-I) was begun. The conversion of hepatic glucose output to hepatic glucose uptake occurred in all three dogs, but the response was delayed. This delay in the onset of effect could be explained by the potential for competitive inhibition of uptake of HDV-I at the hepatic Ashwell receptor by other glycoproteins bearing terminal galactose.[30]

Physiological Mechanism of Action

Mechanism of Action in Insulin-Deficient Rats. The physiological mechanism of action of injectable HDV-I was studied in three groups (n = 5 or 6/group; 250 grams) of streptozotocin/alloxan-induced, insulin-deficient female Sprague–Dawley rats. After insulin deficiency was induced, rats were maintained on daily doses of neutral protamine Hagedorn insulin (Novo Nordisk, Inc., Princeton, NJ) sufficient to maintain blood glucose in the 200- to 300-mg/dl range. The three groups received saline, regular insulin, or HDV-I (1% bound to HDV and 99% free insulin), respectively. Insulin doses were 0.32 IU/250 gram body weight in a 0.2-ml volume, and 0.2 ml saline was administered. Immediately after administering the test doses, ^{14}C-labeled glucose (1.5 grams) was injected intraperitoneally, which would be absorbed into the portal vein to simulate an oral glucose meal. Rats were sacrificed 2 hours postdosing, blood samples for glucose were obtained, and the entire livers were removed and processed to determine the ^{14}C content as an indicator of net glucose uptake. Two hours following treatment, RI-treated rats had a slight increase in the mean blood glucose value from the mean baseline value, which was not statistically significantly different from saline treatment [+25.0 ± 99 mg/dl (or 4.3%) versus +43.7 ± 115 (or 9.3%), respectively] (**Figure 6**). In contrast, the same dose of HDV-I was associated with a marked reduction from baseline in the mean blood glucose value by –193.2 ± 222 mg/dl (or 36.1%), which was statistically significantly different (p = 0.0427) from the mean change in blood glucose following the administration 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). Also, following the administration of HDV-I, there was significantly more radiolabeled glucose in the rat livers (5184 ± 2973 cpm/g) compared to RI (2023 ± 810 cpm/g; p = 0.0308) and saline (1734 ± 461 cpm/g; p = 0.0314) treatments. Despite the considerable variability of the results, the more marked and significant lowering of the mean blood glucose level by HDV-I appeared to be the result of increased hepatic storage of [^{14}C]glucose. Therefore, these data support the hypothesis that HDV-I activates the normal hepatic glucose storage mechanism during a meal, presumably by a direct hepatic action, restoring the normal physiological mechanism of hepatic glucose metabolism while conventional SC insulin does not.

Mechanism of Action in Dogs as Measured by Oral Glucose Tolerance Test. The OGTT was used to study the mechanism of action of injectable HDV-I before and after inducing diabetes in mongrel dogs. Prior to pancreatectomy, four

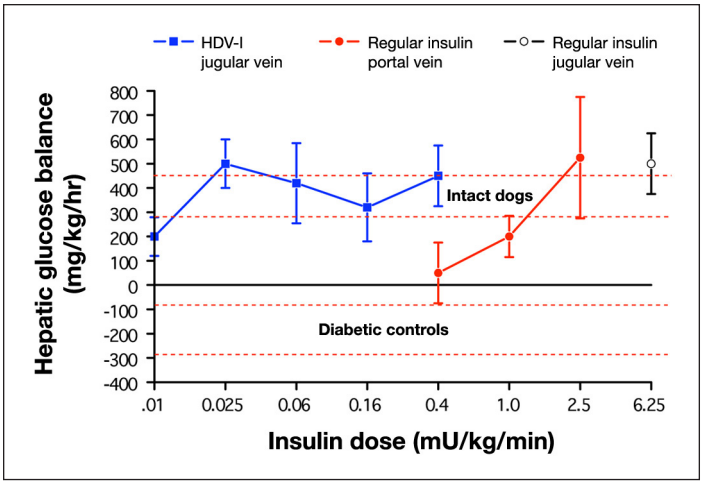

Figure 5. Summary data for **Figure 4.** Mean ± SEM hepatic glucose balance for control intact and control (no insulin) diabetic dogs receiving 0.5 g/kg/hr glucose via the portal vein are shown as areas between two sets of parallel dashed lines. The effect of regular insulin and HDV–insulin infusion via the external jugular vein on hepatic glucose balance in diabetic dogs receiving 0.5 g/kg/hr glucose via portal infusion are depicted as a dose–response (n = 6 each). HDV, hepatic-directed vesicle.

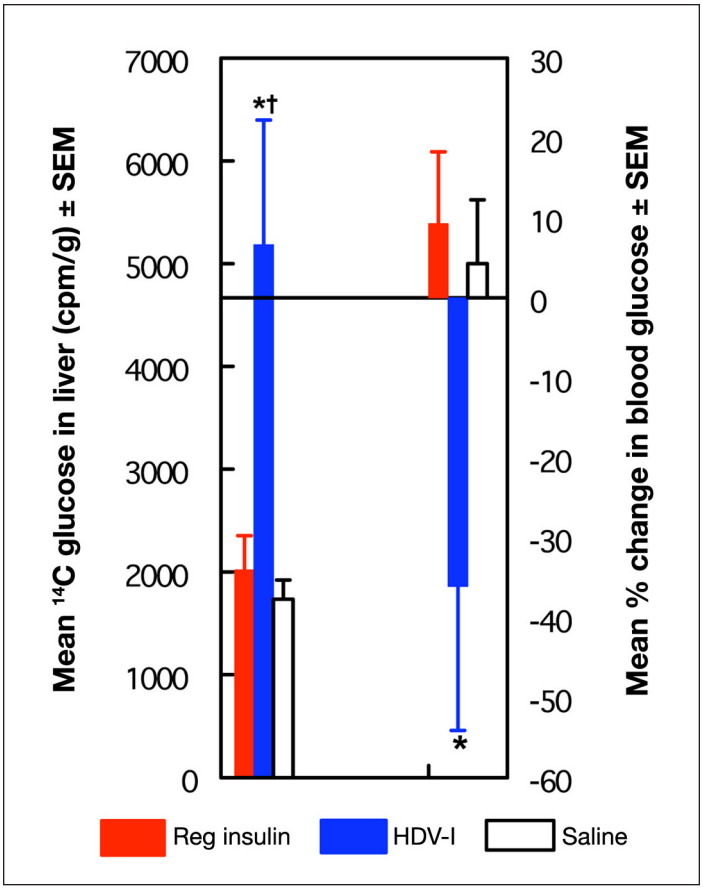

Figure 6. Effect of HDV–insulin, regular insulin, and saline on blood glucose and hepatic [^{14}C]glucose uptake in insulin-deficient rats. n = 5 (saline); n = 6 each (regular insulin and HDV-I). *p < 0.05 compared to the corresponding mean value for regular insulin. †p < 0.05 compared to the corresponding mean value for saline. HDV, hepatic-directed vesicle.

dogs (normal) were trained to drink a glucose solution (1.5 g/kg body weight) in 5 minutes in the morning before receiving their daily meal. Peripheral venous blood samples were obtained via the saphenous vein following a prespecified schedule before and for 3 hours after ingesting the glucose meal. Following pancreatectomy (n = 9), the dogs were allowed to recover and were maintained on insulin therapy so that their morning fasting blood glucose levels were approximately 100 mg/dl. The OGTT was then repeated with both their regular premeal dose of regular insulin and then with the same total dose of HDV-I where the HTM was disofenin and the concentration of HDV was 1 mg HDV/100 IU insulin/ml. The typical dose of regular insulin or HDV-I was 20–40 IU. **Figure 7** shows the comparative OGTT responses in normal dogs and in panreatectomized dogs following insulin therapy with RI and HDV-I. The pancreatectomized dogs received premeal subcutaneous dosing with human recombinant insulin and, after a day of rest, received an equal dose of HDV-I in which 1% of the insulin was targeted to hepatocytes. The OGTT curve for intact (normal) dogs showed the typical rise in blood glucose with a return to baseline by 3–4 hours postmeal. Pancreatectomized dogs receiving regular insulin had a significant elevation of blood glucose above the level seen in intact dogs, followed by a reactive hypoglycemia. The same dogs, receiving the same total dose of insulin as HDV-I, had OGTT curves indistinguishable from the prepancreatectomy curves. In other words, normal glucose tolerance was reestablished with the hepatic targeting of insulin. These results demonstrated that injectable HDV-I was more effective in controlling hyperglycemia and in preventing secondary hypoglycemia during an OGTT than the same dose of RI, suggesting a direct hepatic effect of HDV-I.

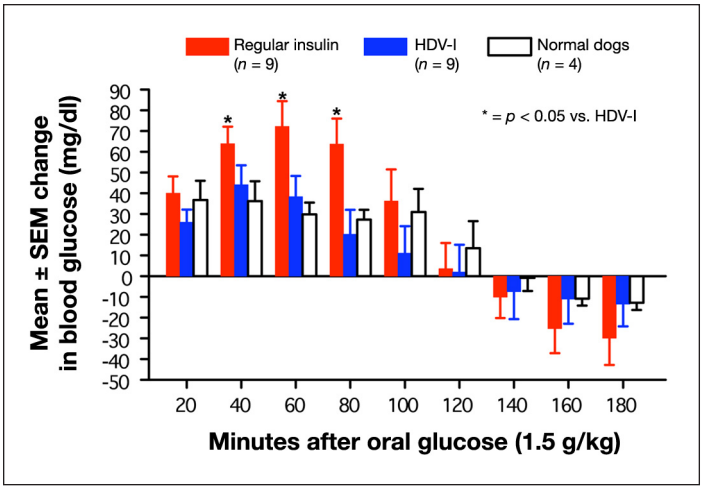

Figure 7. Effect of regular insulin and HDV–insulin in pancreatectomized dogs and saline in normal dogs dosed 15 to 30 minutes before an oral glucose load on the OGTT. Doses of regular insulin and HDV-I were the same (0.15 IU/kg). Data for the curve of normal dogs (n = 4) were obtained before pancreatectomy (n = 9). HDV, hepatic-directed vesicle.

Toxicology Studies

A 28-Day Subcutaneous Repeated-Dose Toxicity Study in Rats. The potential toxic effect of HDV was evaluated in a 28-day study in Crl:CD® (SD)BR rats. HDV (with disofenin as HTM) was administered via subcutaneous injection to three groups of 10 males and 10 females each at dosage levels of 0.6, 1.2, and 2.4 mg/kg/day. No abnormal clinical or clinical laboratory adverse effects and no changes in food consumption, body weight, or histopathological findings were observed. Specifically, no HDV-related effects were observed in the liver (including histopathology) or in any of the gross lesions examined. In addition, no accumulation of HDV was seen in the liver histopathologically.

Genetic Toxicology Studies. The standard battery of three genotoxicity studies, including the Ames assay (or bacterial reverse mutation assay), *in vitro* mammalian chromosome aberration test, and mouse micronucleus test, was conducted with HDV. The results of all three studies were negative. HDV was not mutagenic or genotoxic.

Conclusions

The present results have clearly demonstrated that HDV-I, a nanoparticle with very low toxicity, effectively targets the hepatocytes of the liver specifically and is an effective insulin-replacement treatment in diabetic animal models. Results showed that injectable HDV administered peripherally to hyperglycemic diabetic dogs delivers insulin to the liver efficiently and promotes hepatic glucose uptake with a potency that is at least 100-fold greater than that of the same dose of regular porcine or human recombinant insulin. Results support the hypothesis that HDV-I works by stimulating hepatic glucose uptake and/or glycogen storage in insulin-deficient animals. The ability to target the delivery of HDV-I to the liver reestablishes the liver as a major metabolic modulator of glucose metabolism and is a significant advance in diabetes insulin therapy. HDV is composed of naturally occurring phospholipids and a vitamin that have a generally recognized as safe status. Results of the HDV 28-day toxicity study in rats and the battery of genetic toxicology studies are a further confirmation of its safety. The future of HDV-I will depend on the results of ongoing development, including analytical methods, stability, and receptor-binding studies, and on the results of longer term clinical trials.

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Disclosure:

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