Breaking Away: Proceedings of the 21st American Peptide Symposium
Michal Lebl (Editor)
American Peptide Society, 2009

Oral Delivery of Peptide Hormones: Insulin, Interferon, Growth Hormone and Calcitonin

W. Blair Geho and John R. Lau
SDG, Inc., Cleveland, OH 44106, U.S.A.

Introduction

Peptides and proteins are routinely administered by parenteral injection and their delivery to specific sites in the body are a function of random delivery. Peptide hormone efficacy has been studied in model bio-nano formulations in which peptides are carried to specific cell receptors by means of a bipolar lipid membrane fragment which is "targeted" to receptors by specific receptor target molecules. The leading example is HDV-Insulin (hepatocyte directed vesicle insulin). HDV-Insulin has been shown in animals and humans to be effective in activating hepatocyte insulin responses by both injection and oral routes of administration, at very low doses. HDV-Insulin is in Phase 2b studies in type 2 diabetes mellitus subjects. The same formulation approach has been taken with other peptide hormones such as interferon alfa, calcitonin, growth hormone and IgG antibodies. All of these formulations have been successfully shown to be bioavailable by the oral route of administration. This formulation technology may represent a major advance in developing oral peptide products.

Formulation Description

SDG's Bio-nano formulations use a 20-50 nm bipolar phospholipid membrane fragment as a basic structure to which targeted molecules and RES (reticuloendothelial system) masking molecules such as sialic acid can be attached to give the carrier both receptor specificity and immune cell anonymity HDV is a membrane consisting of lecithin, cholesterol and dicetyl phosphate. Hepatocyte specificity is by means of inserting biotin as a hepatocyte receptor targeting molecule. Human recombinant insulin is attached passively by means of association of insulin with the combined electrical and membrane hydrophobic regions. Large scale manufacturing of cGMP HDV-Insulin has been completed and is suitable for oral formulations by means of a specific complex with gelatin, which is then filled into standard hard gelatin capsules. Without gelatin, the formulation is suitable for injection.

HDV-Insulin for subcutaneous administration is a mixture of 1% of total insulin bound to HDV, with the remaining 99% being non-HDV, or "free" insulin. Oral HDV-Insulin has 100% of the insulin bound to HDV.

The bio-nano cGMP oral HDV-Insulin capsules have extended stability even at elevated temperatures and humidity for six months. Injectable HDV-Insulin has the same stability profile as recombinant human insulin for injection.

Other peptides, such as growth hormone, interferon, and calcitonin may be substituted for insulin, with similar kinetics of attachment to the basic membrane. The peptide-carrier structures can either be targeted or non-targeted. Extended circulation times and hepatic bypass characteristics can be achieved by adding sialic acid to the membrane carrier.

Results and Discussion

Therapeutic peptides and proteins have been administered parenterally because oral delivery did not work: the proteins and peptides were both digested in the gastrointestinal tract and they were also not absorbed across the gut into the portal circulation. HDV is an example of a bio-nano delivery particle that both protects peptides and proteins from digestion, and also facilitates their gut absorption. The effect of subcutaneously injected HDV-Insulin on oral glucose tolerance tests (OGTT) has been studied in dogs[1] and humans [2], and orally administered to type 1 and type 2 [3] diabetes mellitus patients. Results of an OGTT in pancreatectomized dogs is shown in Figure 1 and humans with type 1 diabetes in Figure 2. HDV-I treatment in insulin deficient dogs normalized OGTT, with the blood glucose levels closely approximating the normal control curves of intact dogs. This is in contrast to the abnormal curves of the insulin deficient dogs receiving equal subcutaneous doses of regular, non hepatic targeted insulin. A similar effect on OGTT was observed in the human study. Peripheral plasma insulin levels as well as blood

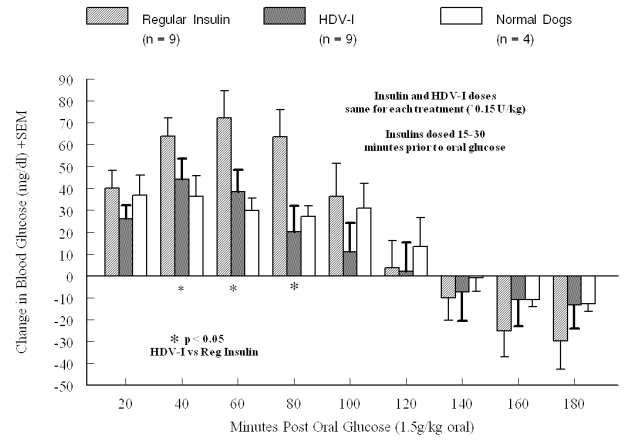

Fig. 1. OGTT in normal dogs and pancreatectomized dogs treated subcutaneously with either regular insulin or HDV-Insulin.

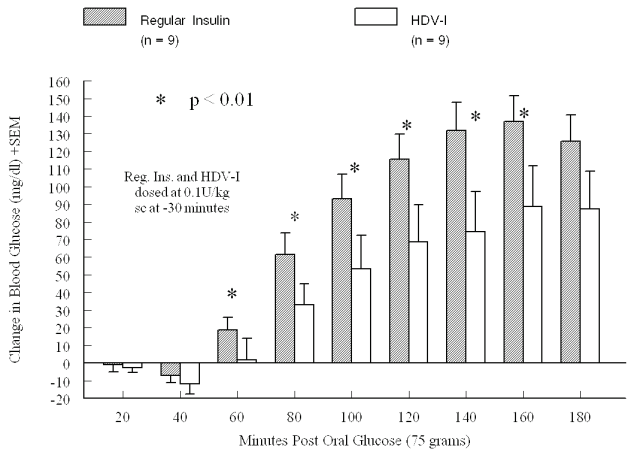

Fig. 2. OGTT in human type 1 diabetic subjects treated with subcutaneously administered regular insulin or HDV-Insulin.

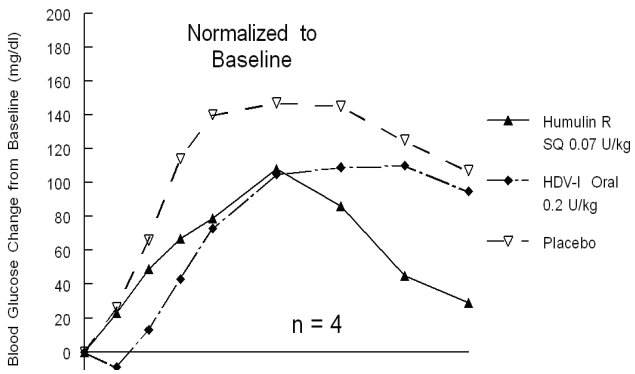

Fig. 3. OGGT in type 1 diabetics treated with subcutaneous regular insulin or oral placebo or HDV-Insulin.

levels of relevant counter regulatory hormones such as glucagon and cortisol were the same for both forms of injected insulin, strongly suggesting that the improved OGTT curves were due to hepatic glucose retention and not a peripheral effect of insulin on fat and muscle tissues.

HDV-Insulin (100% of insulin bound to HDV) was then formulated into an oral dose form by complexing aqueous HDV-Insulin with pharmaceutical grade gelatin powder. The resultant dry granulation was put into small hard gelatin capsules containing 5 IU human recombinant HDV-Insulin. In an open label crossover trial in which subcutaneous regular insulin at 0.07 IU/Kg body weight was compared to 0.1 IU Oral HDV-Insulin/ kg body weight, administered 30 minutes prior to meals to type 1 diabetes mellitus patients resulted in similar significant reductions for both forms of insulin in the post-prandial area under the curve (AUC) analyses (Figure 3) compared to placebo control. Type 2 diabetics [3] on background metformin therapy were administered Oral HDV-Insulin dosed over a dose range of 0.05 to 0.4 IU HDV-insulin/kg body weight, following a baseline OGTT with metformin alone and an oral placebo. All responses were significantly different from placebo treatment (Figure 4). The "flat" dose response was predicted by earlier animal studies. The "flat" dose response suggested that a 5 IU oral HDV-Insulin dose would be sufficient for human diabetes subjects weighing 50-120 kg. This 5 IU Oral Insulin dose form in a size 2 gelatin capsule is now in a large double-blind phase 2b clinical trial in type 2 diabetes mellitus subjects. Phase 2a data from a number of studies all confirm this remarkable oral activity. The success of oral delivery with human recombinant insulin suggested using similar

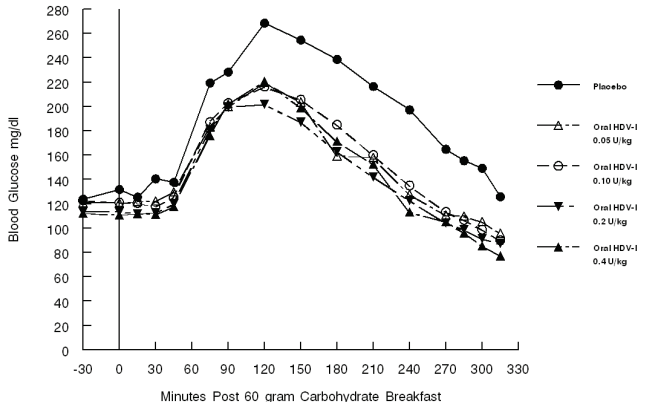

Fig. 4. Post-Breakfast Blood Glucose levels in 6 type 2 diabetics on metformin treated with oral placebo capsules or increasing doses of oral HDV-Insulin.

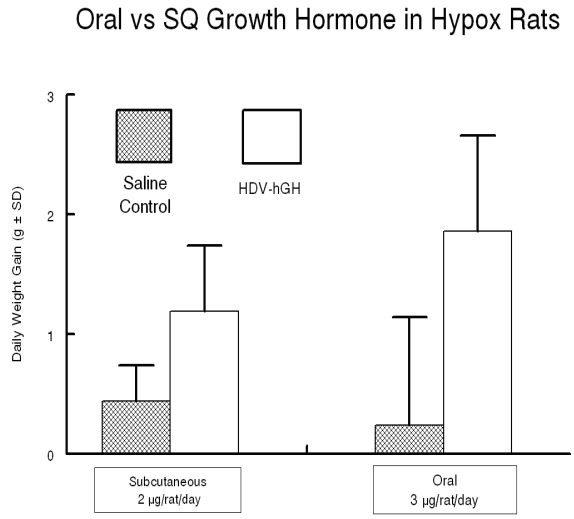

Fig. 5. Effect of Oral delivery of hGH in 6 Hypox rats on weight gain.

delivery particles to deliver other materials to the liver by the oral route. Interferon alfa has been formulated with HDV for hepatocyte delivery. Pre-clinical studies in mice in which PKR RNA markers that are responsive to interferon alfa have been used as indicators of hepatocyte delivery of interferon. Equal doses HDV-Interferon alfa were administered by intravenous and oral routes. The RNA markers of interferon activity were equally stimulated by HDV-Interferon administered by both routes of administration.

HDV has also been used, in a similar manner, to carry human growth hormone (hGH), administered both orally and parenterally at 1 and 3 ug/kg body weight per day, to hypophysectomized rats for a week to determine growth hormone efficacy on growth. HDV-hGH stimulated of the rats significantly compared to placebo treated rats (Figure 5).

The bio-nanoparticle delivery system exemplified by HDV is a versatile vehicle for both parenteral and oral delivery of a variety of peptides and protein hormones. The pharmacodynamic efficacy of several therapeutic proteins has been shown to be similar by both parenteral and oral administration. The SDG bio-nanoparticle delivery system is a potential mechanism for the delivery of many protein and peptide base pharmaceutical products.

Acknowledgments

These studies were funded by SDG, Inc., and AMDG, Inc., both of Cleveland, Ohio, and Diasome Pharmaceuticals, Inc., Conshohocken, Pennsylvania.

References

1. Geho, B., Lau, J., Rosenberg, L. *Diabetes* **57**(Suppl 1):A126 (2008).
2. Davis, S.N., Geho, B., Tate, D., Galassetti, P., Lau, J., Granner, D., Mann, S. *J. Diabetes Complications* Sep-Oct; **15**(5):227-233 (2001).
3. Schwartz, S., Geho, B., Rosenberg, L, Lau, R., *Diabetes* **57** (Suppl 1): A127 (2008).