NOVEL LOW MOLECULAR WEIGHT HEPARIN-INSULIN CRYSTALLINE FORMULATIONS FOR IMPROVED NON-INVASIVE INSULIN DELIVERY

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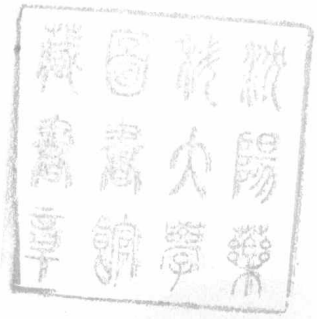

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THESIS

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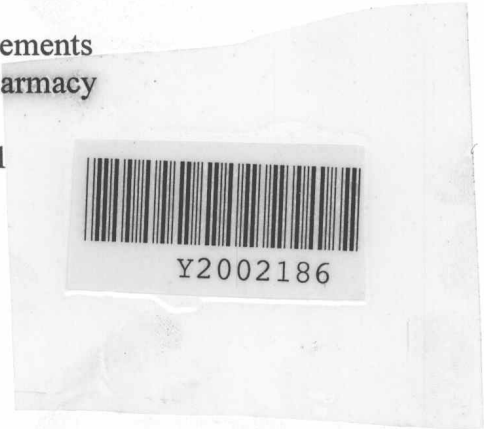

This thesis is dedicated to my husband, Xiaohui Mei, my parents, Jingyu Zu and Yuhua Fu, and my brother Yanlei Zu, without whom it would never have been accomplished.

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YZ

LIST OF ABBREVIATIONS

AAC	Area above Curve
ACC	Animal Care Committee
ATIII	Antithrombin III
DKA	Diabetic Ketoacidosis
ESRD	End-Stage Renal Disease
GAG	Glycosaminoglycan
GDM	Gestational Diabetes Mellitus
GlcN	D-Glucosamine
HHS	Hyperosmolar Hyperglycemic State
HS	Heparan Sulfate
IDDM	Insulin Dependent Diabetes Mellitus
Idu	L-Iduronic Acid
LM	Light Microscopy
LMWH	Low Molecular Weight Heparin
MODY	Maturity-Onset Diabetes of the Young
MWCO	Molecular Weight Cut-Off
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NPH	Neutral Protamine Hagedorn
PBS	Phosphate Buffer Saline
RER	Rough Endoplasmic Reticulum
SEM	Scanning Electron Microscopy
SLF	Simulated Lung Fluid

SUMMARY

Diabetes mellitus is a metabolic disorder characterized by fasting hyperglycemia and abnormalities in the metabolism of carbohydrates, proteins, and lipids. These abnormalities result from a deficiency in the secretion and/or action of insulin, and are associated with a variety of life-threatening short- or long-term complications. As the sixth leading cause of death in the United States, diabetes mellitus affects approximately 6% of the U.S. population with an annual economic burden approaching $90 billion. It is one of the most common, serious, and costly health problems in the United States.

Since its discovery by Banting and Best in 1921, insulin is the cornerstone for the treatment of diabetes mellitus. Due to its protein nature and subsequent rapid degradation following oral administration, insulin is typically administered as daily subcutaneous injections. However, the pain and fear of needles contribute to poor patient compliance, resulting in improper disease management. Consequently, extensive efforts have been devoted to the development of various non-oral non-invasive routes of administration as alternatives to injection. Although insulin therapy alone establishes normal blood glucose levels, a more complex and difficult issue that has not been addressed is the treatment of the long-term vascular complications associated with diabetes. It has been established that subcutaneous administration of heparin and other glycosaminoglycans (GAGs) ameliorate diabetic vascular complications, although the exact mechanism is unclear.

It was the intent of this work to combine the advantage of insulin with the advantage of glycosaminoglycans, and characterize, evaluate, and develop a new heparin-insulin complex that will (1) stabilize blood glucose levels (2) ameliorate the chronic complications associated with diabetes and (3) provide therapeutic levels of both agents across parenteral mucosal membranes.

Insulin, with an average molecular weight of 5,700 daltons, is a polyamphoteric peptide drug with an isoelectric point of 5.3-5.4. The potential of insulin to complex and crystallize in a variety of forms has been utilized in the development of formulations that retard the action of insulin after injection. Found exclusively in mast cells, heparin is a polydispersed, highly sulfated, linear glycosaminoglycan with a molecular range of 12,000 to 20,000 daltons. Low molecular weight heparin (LMWH) is a fragment of heparin produced by either fractionation or depolymerization, with an average molecular weight of 4,000 to 6,500 daltons. The ability of heparin to interact with alkaloids, amines and proteins is well known, and this association is responsible for the many biological activities of heparin and related compounds. Therefore, it is expected that insulin would exhibit electrostatic and/or hydrophobic affinity for poly-anionic heparin, at least at acidic condition.

It is known that heparinic acid interacts with basic or amphoteric compounds, and that these complexes demonstrate enhanced absorption across mucous membranes.

Therefore it is speculated that complexes of insulin and LMWH might also show enhanced absorption via various non-oral non-invasive alternative routes, including but not limited to pulmonary and nasal administration. The hypotheses of this study are that insulin will form non-covalent macromolecular complexes with LMWH, and that these LMWH-insulin complexes will provide therapeutic plasma levels of insulin following pulmonary and nasal administration. Although beyond the scope of the present work, it is postulated that these novel insulin formulations can ameliorate diabetic vascular complications.

In this study, the novel LMWH-insulin crystalline complexes were isolated from an acidic environment. A number of the physicochemical properties of these complexes were determined, including the crystal structure by scanning electron and light microscopy, and the stoichiometry of the LMWH-insulin crystal. Particle sizes of different weight ratios LMWH-insulin crystalline suspensions were measured and the effect of pH on the stoichiometric ratio LMWH-insulin established. Scanning electron and light micrographs revealed that the novel LMWH-insulin complexes exist as monoclinic LMWH-insulin crystals. The stoichiometric ratio of the LMWH-insulin crystalline complexes at pH ~ 3 is approximately 85.1:14.9 insulin:LMWH w:w. The mean particle size of the stoichiometric ratio LMWH-insulin crystalline suspension is 743.0 ± 20.9 nm. The stoichiometric ratio LMWH-insulin complexes are essentially zinc-free below pH 4, and completely solubilize at pH ≥ 6.

The hypoglycemic effects of these novel LMWH-insulin crystalline suspensions were measured following pulmonary and nasal administration, and compared to the hypoglycemic effects of two clinical insulin formulations, NPH (neutral protamine Hagedorn) insulin suspension and REGULAR insulin solution. Following intratracheal and nasal instillation, novel LMWH-insulin formulations significantly increased the systemic absorption of insulin and produced a sustained hypoglycemic effect. The LMWH-insulin suspension with excess LMWH (LMWH:insulin 80:20 w:w) is nearly twice as apparently efficacious and five times as apparently potent as the commercial NPH insulin suspension following intratracheal instillation. Following nasal instillation, the LMWH-insulin suspension with excess LMWH (LMWH:insulin 80:20 w:w) is approximately twice as apparently efficacious as the NPH insulin suspension.

At an insulin dose of 1.0 IU/kg, although there was no significant difference between the hypoglycemic effect produced by the LMWH-insulin stoichiometric ratio suspension and the commercial REGULAR insulin solution following intratracheal instillation. The LMWH-insulin suspension with excess LMWH (LMWH:insulin 80:20 w:w) showed a significantly greater hypoglycemic effect than both the LMWH-insulin stoichiometric ratio suspension and REGULAR insulin solution (ANOVA multiple comparison, $p < 0.10$). It is speculated that the presence of a large excess of LMWH facilitates insulin absorption across the mucous membranes of the lung from the LMWH-insulin suspension with excess LMWH. The commercial NPH insulin suspension

showed the least hypoglycemic effect among all insulin formulations ($p < 0.01$), presumably due to its largest particle size (~ 10 μm) and poor solubility in actual lung fluid (NPH solubility in simulated lung fluid ~ 0.57 mg/ml).

At an insulin dose of 5.0 IU/kg, the LMWH-insulin suspension with excess LMWH (LMWH:insulin 80:20 w:w) showed a significantly greater hypoglycemic effect than both NPH insulin suspension and REGULAR insulin solution (ANOVA multiple comparison, $p < 0.01$) following nasal instillation. It is again postulated that the excess LMWH in the LMWH-insulin suspension facilitates insulin absorption across nasal mucous membranes. One explanation for the significantly greater hypoglycemic response produced by NPH insulin suspension as compared to REGULAR insulin solution is that the less viscous REGULAR insulin solution is removed rapidly from the nasal cavity by the nasal mucociliary clearance system.

Thus it is concluded that novel crystalline LMWH-insulin formulations provide a promising way for the non-invasive insulin delivery, and at the same time offer the potential for the prevention and treatment of diabetic vascular complications.

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1. INTRODUCTION

1.1 Statement of Problem

1.1.1 Social, racial and economic impact of diabetes mellitus

An estimated 16 million Americans or approximately 6% of the U.S. population are affected by diabetes mellitus, and each year more than 625,000 new diabetes cases are diagnosed (American Diabetes Association, 1996). The prevalence and incidence of diabetes increases yearly with minority and elderly populations disproportionately affected. The age-adjusted prevalence of diabetes was 16% higher and incidence of diabetes 49% higher in 1994, as compared with the same indicators in 1980 (Centers for Disease Control and Prevention, 1997). African-Americans and other racial minorities, as compared to Caucasians, are more vulnerable to diabetes and its long-term complications. The prevalence of diabetes among African-Americans is 12.5% and 13.7% among Mexican-Americans (Harris et al., 1998). Diabetes prevalence and incidence increases with age, with nearly 11% of the U.S. population between age 65 to 74 exhibiting symptoms of diabetes mellitus. Furthermore, 50% of all new cases of diabetes are diagnosed in the elderly (American Diabetes Association, 1996).

Diabetes mellitus is the sixth leading cause of death and disability in the United States. In 1994, diabetes contributed to the deaths of over 182,000 individuals (Centers for Disease Control and Prevention, 1997). In addition to causing chronic hyperglycemia, diabetes produces detrimental effects on other body organs. Diabetes and its associated complications are the leading cause of adult blindness, end-stage renal