EQUIDYNE SYSTEMS, INC.

TECHNICAL REPORT TR-01-001

Retention of structural/potency characteristics of Lantus Insulin, AKA Insulin Glargine, or HOE901

ANALYTICAL INVESTIGATIONAL STUDY
EQUA-004

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I. INTRODUCTION AND PURPOSE

The Equidyne Systems, Inc. Injex device is an FDA-approved (for sub-cutaneous injections) needle-free delivery device which utilizes a mechanical pressure technology to propel 0.05-0.3 ml of solution through a 0.006" orifice under a pressure of ~3000 psi. Previously, trials have been performed by various pharmaceutical companies and Equidyne to determine whether compounds will be 'sheared', inactivated, or otherwise damaged due to the pressure of injection. The previous studies performed by Equidyne (Protocols EquA-001 and EquA-003) demonstrated no detectable effect on various insulins including Humalog, Humulin-R, Humulin L, Humulin U, NPH, Iletin, Velosulin, and Novolin, as well as model compounds spanning a molecular weight range of 700 to ~1 million daltons.

Since that time, Lantus® insulin (Aventis Pharmaceuticals, Inc.) has been approved by the Food and Drug Administration (FDA) for the treatment of adults and children with type I diabetes mellitus, and adults with type 2 diabetes who require long-acting insulin for control of hyperglycemia. This new insulin is produced by recombinant DNA technology and differs from endogenous insulin by three amino acids: asparagine is substituted for a glycine residue at position A21 of the A-chain and two arginine residues are added at positions B31 and B32 of the B chain. Lantus is of interest as the molecular structure causes a precipitation in the subcutaneous tissue after injection. This precipitation delays absorption, the pharmacokinetic outcome of which is a long distribution and elimination phase with no pronounced peak.

The present study was designed to determine whether this new insulin retains its molecular structure and/or potency post-injection with the Injex™ needle-free delivery device. Comparisons to delivery via a standard syringe as well as a control retention were performed.

II. SCOPE

The methods, observations, results, and conclusions in this report are applicable to evaluation of molecular damage to Lantus insulin as a result of injection via the Injex in comparison to a control and a standard needle syringe.

III. ACRONYMS AND DEFINITIONS

- RIA: radioimmunoassay based on competitive inhibition of labeled substrate vs. native substrate for binding by a monoclonal or polyclonal antibody.

IV. PERFORMED BY

This study was performed, managed, and analyzed by Mark J. Sarno, Scientific Consultant to Equidyne Systems and Founding Partner, Vision Biotechnology Consulting.

V. REFERENCES

Reports: EquA-003 (TR105) and EquA-001

VI. EQUIPMENT, MATERIALS, AND METHODS

STUDY SYNOPSIS

The study is designed as an in-vitro test using an immunometric method to indicate outcome.

EQUIPMENT

Equidyne Injex 0.3 ml injector, reset box, appropriately sized ampules, and small vial adapters were required for this study. In addition, Becton-Dickinson 0.5 ml syringes and 28 gauge needles were required.

SUMMARY OF METHODS

A. Materials

Lantus insulin (insulin glargine or HOE901) was obtained courtesy of Aventis Pharmaceuticals. Lot H007 (exp. 4/2002) was used for this study. The nominal concentration (label claim) was 100 Units/milliliter (U/ml). The material is supplied as a clear solution with no visible contaminants.

B. Measurement method

Lantus insulin samples were measured by radioimmunoassay (RIA). The assay utilizes guinea pig antiserum specific for insulin as the primary antibody and radioiodinated recombinant human insulin as tracer. A second antibody specific for guinea pig immunoglobulin is used to precipitate immune complexes. Each sample was tested in duplicates. Testing was performed by the Esoterix unit of Endocrine Sciences (Calabasas, CA). All samples required dilutions in order to achieve concentrations within the quantitative linear range of the assay (0-60 µU/ml)

C. Test injections

For each delivery method 6 deliveries were performed, i.e. 0.3 ml was delivered 3 times into two Type I glass receptacles by a Becton-Dickinson 0.5 ml syringe fitted with a 28 gauge needle, and another 6 aliquots of 0.3 ml were delivered into two Type I glass receptacles by the Injex 0.3 ml injector. A pre-delivery retention was kept as a control. The Injex and syringe test aliquots were then compared to the retention control using the immunoassay method described above.

D. Statistical methods

Standard statistical methods were employed to compare the results (U/ml) from the Injex, syringe, and control solutions. Means and standard deviations (SD) are reported. Additionally, insulin values from the Injex, syringe, and control were compared parametrically using the Students-t test. Differences between delivery methods and vs. the control are reported. $P<0.05$ was considered significant.

VII. RESULTS

Table 1 displays the results from the immunoassay analysis. Means and standard deviations (SD) are reported for the Injex device, the syringe, and the control retention.

TABLE 1: Immunoassay results by injection device, or control

Device/Control	*Mean (U/ml)*	*SD*	*p vs. Control*
Control	52.0	2.83	
Injex	49.5	2.12	0.4226
Syringe	50.5	2.12	0.6094

This new insulin had not been previously tested in the insulin radioimmunoassay. The results suggest that the antibody utilized in this assay is only 50% cross-reactive as the expected result for the control was ~100 U/ml. However, the results are internally consistent, i.e. the actual results from the Injex and the syringe are being compared to the actual results for the control, not the expected concentration.

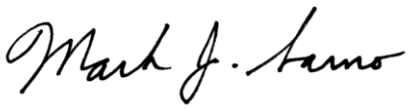
Thus, the results demonstrate no loss in Lantus insulin potency after injection by either the Injex device or the syringe in comparison to the control. The results of the Students t-test are not significant, i.e. $p>0.05$.

VIII. CONCLUSIONS

This in-vitro study demonstrates complete retention of molecular identity of Lantus insulin injected via the Injex. The immunoreactivity was retained, thus suggesting that epitope moieties were maintained intact despite the high pressures of injection utilized by the Injex methodology. It is a logical extension that if epitopes are maintained intact, that the overall primary and secondary structure of the molecule is likely intact. To explain further, molecular epitopes for peptide/protein molecules are generally on the order of tens of amino acids. Since Lantus insulin is a dipeptide on the order of 50 amino acids, it is unlikely that any shearing can have occurred.

Further, protein epitopes are generally required to be in a three dimensional conformation consistent with the original antigen used to produce and select antibodies. Since there is no loss of immunoreactivity in this study (compared to the control), the three dimensional conformation (tertiary structure) of the molecule is also maintained after injection. It is therefore logical to conclude that structural damage (cleavage of the disulfide bonds or unwinding of the tertiary structure) has not occurred as a result of injection.

Respectfully submitted,



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