Comparison of *in vitro* and *in vivo* insulin bioidentity assays to monitor the quality of insulin products

JuMe Park[1], Hyunsu Shin[1], Sujata Bupp[2], Susmita Ghosh[1], Mamatha Garige[1], Steven Kozlowski[2], Carole Sourbier[1]
1. CDER/OPQ/OBP/DBRRI ; 2. CDER/OPQ/OBP/DBRRII

Abstract

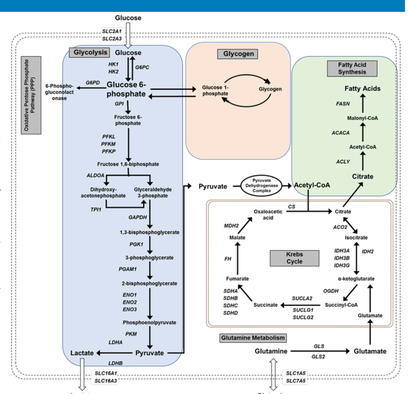
Over 100 million individuals manage their diabetes with insulin products daily and rely on the pharmaceutical quality of these products to regulate their blood glucose. Since March 23rd, 2020, insulin products are regulated as biologics. Under the regulations for Biologics License Application review, it is expected that the potency of the insulin products and of their associated biosimilars will be assessed quantitatively in a cell-based assay or bioassay that, ideally, represents the product mechanism of action. Currently, the USP is recommending an *in vivo* rabbit bioidentity test for the assessment of the biological activity, or bioidentity, of insulins (USP <121>). However, reduction in animal experiments is a worldwide goal for many and it is likely that *in vitro* bioassays will be submitted for assessing the bioidentity of insulin products. Our goal is to evaluate how to best transition testing of insulin products from *in vivo* assays to *in vitro* bioassays. With this study, we are planning to compare the potency and kinetics of stressed and unstressed insulin products using both the USP Rabbit Blood Sugar Method and an in-cell western *in vitro* bioassay previously publicly described. Data that will be collected include: effect on glucose metabolism (including monitoring of the full activation of the insulin receptor), dose linearity, percentage coefficient of variation, mean value, maximum effect, minimum effect, standard deviation, as well as practicality, cost and time. Comparison of these parameters will facilitate the transition of insulin bioassays from *in vivo* to *in vitro* and will support the assessment of prospective *in vitro* bioassays to monitor the quality of insulin products.

FDA Mission Relevance

Comparison of in vitro and in vivo bioidentity assays will facilitate the transition of insulin bioassays from *in vivo* to *in vitro* and will support the assessment of prospective *in vitro* bioassays to monitor the quality of insulin products.
This project is responsive to the goals and mission of the US FDA and CDER and is in alignment with the Office of Pharmaceutical Quality Strategic Plan: objective 2.3. "Identify and continue to evolve decision-making tools and resources that enable effective risk-based decisions through best practices, analytics and research findings."

Introduction

Binding of human insulin or insulin analogs to the human insulin receptor (hIR) induces the auto-phosphorylation and activation of hIR which induces a signaling cascade leading to the uptake of glucose by the cells. The utilization of glucose will be dependent of the cell type and may affect glucose, glycogen or lipid metabolism.



Introduction

- Approximately 34.2 million people in the US have diabetes
- Since March 23rd, 2020, insulin products are regulated as biologics. Under the regulations for Biologics License Application review, it is expected that the potency of the insulin products and of their associated biosimilars will be assessed quantitatively in a cell-based assay or bioassay that, ideally, represents the product mechanism of action.
- USP is recommending an *in vivo* rabbit bioidentity test for the assessment of the biological activity, or bioidentity, of insulins (USP <121>). However, reduction in animal experiments is a worldwide goal for many and it is likely that *in vitro* bioassays will be submitted for assessing the bioidentity of insulin products.
- Since February 2021, USP added an in vitro bioidentity test for the assessment of the bioidentity of insulins: the in-cell western assay.
- With this study, we will compare the potency and kinetics of stressed and unstressed insulin products using both the USP Rabbit Blood Sugar Method and the USP in-cell western *in vitro* bioassay previously publicly described. Data that will be collected include: effect on glucose metabolism (including monitoring of the full activation of the insulin receptor), dose linearity, percentage coefficient of variation, mean value, maximum effect, minimum effect, standard deviation, as well as practicality, cost and time.

Materials and Methods

The Rabbit Blood Sugar Method (based on USP <121>)

- 2 samples solutions can be tested against USP standard solutions
- 8-24 animals are required per batch test

USP insulin standards will be subjected to various stressors. The stressed samples will be tested against unstressed standards for comparison of the effect of stress on the potency and kinetic of insulins. Stressors will consist of shaking or temperature variations (freeze-thaw).

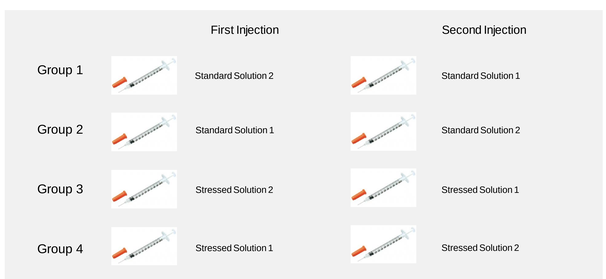


Figure 1. Illustration of the rabbit blood sugar method to monitor insulin products bioidentity.

Dextrose content of the blood specimens will be immediately assessed using a portable glucometer. Potency will be calculated following the USP <121> protocol.

Materials and Methods

In vitro in-cell western assay (based on USP <121>)

Binding of human insulin or insulin analogs to the human insulin receptor (hIR) induces the auto-phosphorylation of its kinase domain which is necessary for kinase and receptor activation. Quantification of insulin-induced auto-phosphorylation of the hIR is an appropriate downstream read-out for insulin and insulin analogs biological activity. A Chinese hamster ovary (CHO) cell line over-expressing the hIR forms the basis of this cellular in vitro assay. Auto-phosphorylation of the hIR is visualized with specific primary antibodies in combination with fluorescent labeled secondary antibodies. A simultaneous cell DNA staining (with a different wavelength than the secondary antibody) enables normalization of the results according to the cell number per microplate well. The final detection is performed with a fluorescent compatible plate-reader (c.f. **Figure 3**).

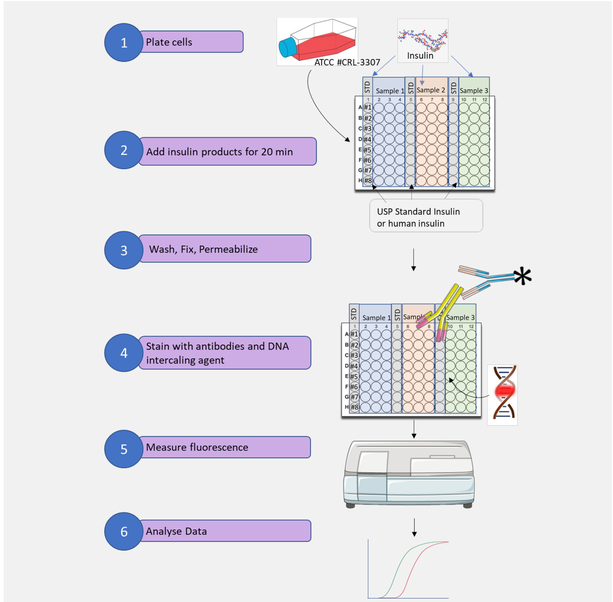


Figure 3. Brief illustration of the in-vitro in-cell western assay to monitor insulin products bioidentity

For each well of each assay plate, we will:
• Normalize the assay signals detected from the secondary antibody that is bound to the primary antibody with the signals from the fluorescent dye for the cell DNA staining.
• Generate a dose-response curve by plotting the processed signal responses from each set of Diluted standard solutions or Diluted sample solutions from one assay plate against their concentrations on a logarithmic scale using a four-parameter logistic (4-PL) constrained model.

Real-time mouse glucose monitoring model

A chemically induced hyperglycemic mouse model will be developed to assess the potency and kinetics of stressed and unstressed insulin with real-time telemetry.

- An implantable transmitter is surgically placed in the carotid of the mice
- Hyperglycemia induced with streptozocin
- Transmitter signals are converted into physiological parameters: blood pressure, temperature, heart rate, and blood glucose level
- Administration of stressed and unstressed insulin are administered, and real-time physiologic changes are continuously recorded

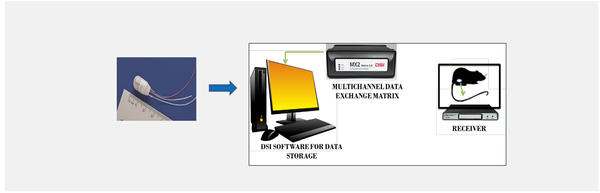


Figure 4. Illustration of the real-time mouse blood sugar method to monitor insulin products bioidentity.

Conclusion

- To best transition testing of insulin products from *in vivo* assays to *in vitro* bioassays, we will assess the robustness of the USP Rabbit Blood Sugar Method, the USP in-cell western *in vitro* bioassay and the real-time mouse glucose monitoring model.
- Potency of unstressed and stressed insulin products will be assessed in parallel using the three different models.
- Data will be analyzed following USP <111> and USP <121> recommendations
- Additional orthogonal *in vitro* approaches representative of insulins mechanism of action will be also developed to further support the transition of insulin bioassays from *in vivo* to *in vitro*.
- This project will support the assessment of prospective *in vitro* bioassays to monitor the quality of insulin products.

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