A COMPARATIVE STUDY OF THREE BRANDS OF BIOSYNTHETIC HUMAN INSULIN

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ABSTRACT

Objective: To compare the activity of three brands of regular biosynthetic human insulin (BHI) in healthy normal subjects with the glucose clamp technique.

Design: A comparative study.

Place and Duration of Study: CMH Lahore from May 2007 to July 2007.

Patients and Methods: Thirty healthy normal male volunteers who met the inclusion criteria were tested. Each insulin preparation was sequentially infused through intravenous route at 0.02 U/kg/h for 2 hour, 0.032 U/kg/h for 2 hour and finally at 0.05 U/kg/h for 2 hours. A simultaneous 25% glucose infusion was maintained and the amount the glucose consumed was calculated. All subjects were studied on three different occasions with the three brands of insulin. Blood glucose was monitored at regular intervals and its level was kept constant (clamped) at a baseline. The amount of glucose infused was calculated for each insulin dose for the three brands of BHI.

Results: Mean values of glucose administered during the six hour infusion period for the three brands ranged between 162 to 190 g. The mean glucose consumed with 0.02 units/kg/hr of Zansulin, Actrapid and Humulin were 26.7gm, 30.6gm and 31.2gm respectively. Similarly the mean glucose consumed with 0.032 units/kg/hr of Zansulin, Actrapid and Humulin were 53gm, 59.7gm and 62.1gm respectively and a similar pattern of glucose consumption was observed for insulin dose of 0.05 units/kg/hr. Glucose administered during the 6 hour infusion differed significantly among the three brands of insulin, ($p < 0.05$).Post hoc test for multiple comparison showed significantly more glucose consumption with Humulin R and Actrapid as compared to Zansulin R (p value <0.05).There was no significant difference in biological activity of Humulin R and Actrapid (p value >0.05)

Conclusion: Humulin R and Actrapid had greater biological activity as compared to Zansulin R when glucose consumption was measured for each using glucose clamp technique.

Keywords: Biosynthetic Human Insulin, glucose clamp technique

INTRODUCTION

The three brands of regular biosynthetic human insulin (BHI) were compared. They are manufactured by Lilly Egypt. 6 October city Cairo Egypt, Novo Nordisk A/S, Denmark and Zafa pharmaceuticals (Private) Limited Karachi. Company representatives of the three brands were invited to review their products. Representatives of all the three companies indicated that problems existed when switching from one brand to the other. These problems included increase in dosage

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requirements and alteration in blood sugar levels. Furthermore, there were concerns regarding manufacturing practices and quality of the products. These reports, although not supported by published literature provided the initiative for this study. In this study the efficacy of the three brands were compared. In addition the study was so designed that the results can be reproduced and the products compared in the office settings of any practicing physician.

A stepwise increasing insulin infusion protocol was used. Blood glucose was maintained at pre-insulin infusion values using the method described by De Fronzo et al [1]. This technique places the blood glucose concentration under the investigator's control and has been called the glucose clamp technique. Keeping the subject normoglycemic has the advantage of eliminating the counter regulatory hormone response to hypoglycemia as a complicating factor. The amount of glucose that had to be infused to maintain normoglycemia gave a direct measure of insulin activity and allowed a quantitative comparison between the three brands of BHI. We report here that using this technique in thirty subjects we were able to show a significant difference between the three brands of regular BHI.

PATIENTS AND METHODS

The study was carried out at CMH Lahore from 2nd May 2007 to 31st July 2007. Thirty healthy male subjects, aged 24-34 years, with body mass index in the range of 18.5-24.9 were selected by convenient sampling method. They had normal fasting blood glucose levels and were not on any medication. There was no family history of diabetes mellitus, hypertension and obesity. All were non smokers and their lipid profiles were within normal range according to National Cholesterol Education Program guidelines. All gave informed consent. Each subject was studied on three occasions with different brands of regular BHI with a wash out period of one week.

Materials:

The brands used were Humulin R (Lilly Egypt. 6 October city Cairo Egypt), Actrapid (Novo Nordisk A/S, Denmark, United Kingdom) and Zansulin R (Zafa pharmaceuticals (Private) Limited Karachi).

Instruments:

All calculations were made with the help of a computer. Blood glucose was measured by One Touch brand of Glucose Meter-manufactured by Life Scan a Johnson and Johnson Company, Milpitas, California.

Protocol:

Subjects fasted from 10 pm on the evening before the study. They were on unrestricted carbohydrate diet for at least three days before the study. At 9 am three cannulas were passed. One in a forearm vein for intermittent sampling of the blood glucose second in the contra lateral antecubital vein for infusion of glucose and the third one also in the contra lateral forearm vein for insulin infusion. The subjects remained at rest for 1 hour. At 10 am an infusion of insulin in saline was started, at 0.02 U/ kg body weight/ h; after 2 hour the infusion rate was increased to 0.032 U/kg/h and after another 2 h to 0.05 U/kg/h. All insulin infusions were controlled by a JMS infusion pump OT 701 JMS Co limited Tokyo Japan. The infusion was stopped at 4 pm and the experiment terminated.

Blood glucose level was maintained at 5 mg/dl below the pre-insulin infusion level. The glucose was infused as 25% solution. Glucose infusion was also controlled by a JMS infusion pump. The computation for the periodic adjustments in the glucose infusion was made every 5 minutes. The computation

has two components that, for convenience, have been designated as a "volume" component and a "metabolic" component. The formula for computing the periodically adjusted infusion rate is Si= SVi+SMi.

Where Si is the setting of the infusion pump at time i; SVi, that portion of the setting needed for the volume component; and SMi, that portion of the setting needed for the metabolic component. SVi is calculated by the formula:

$$SVi = \frac{(Gd - Gi) \times 10 \times (0.19 \times \text{body wt})}{Ginf \times 15}$$

The individual components of this formula are: (Gd – Gi) x10 x (0.19 x body wt) equals the total body glucose deficit or excess in milligrams where Gd is the desired plasma glucose concentration (mg/dl); Gi the actual plasma glucose concentration at any time, i: the multiplication by 10 converts plasma glucose concentration from milligrams per deciliter to milligrams per liters; Ginf is the glucose concentration in the infusate in milligrams per milliliter and converts the glucose dose from milligrams to milliliters of infusate.

The division by 15 is based on a decision to carry out the correction for the volume component over a 15 minute period because the intravenously infused glucose required time for distribution in the total glucose space. The division by 15 converts the computed infusion rate from milliliters to milliliters per minute.

The setting for the metabolic component of the infusion rate is calculated as an iterative procedure. SMi = SMi-2 x FMi x FMi-1.

Where SMi-2 is the metabolic component, calculated two iterations (10min) previously and FMi = Gd/Gi.

Where Gd is the desired and Gi the glucose concentration at any time i. FMi is a dimensionless correction factor that compensated for the error in the plasma glucose concentration. FMi-1 is the FMi calculated one iteration (5 min) previously.

The final formula for computing the adjusted infusion rate is:

$$Si = \frac{(Gd-Gi)\times 10\times(0.19\times \text{body wt})}{Ginf \times 15} + (SMi-2)\times(Gd/Gi)\times(FMi-1)$$

The glucose infusion is not begun until 4 min after attaining the desired glucose concentration (5 mg/dl below the pre-insulin infusion level) and is empirically set at 2.0 mg/kg-min. It is increased at 10 min to 2.5 mg/kg-min. The servo correction formula to modify the infusion rate is first used when the 10 min blood glucose concentration becomes available. For the computation of the infusion rates based upon the 10 and 15 min samples the assigned SM1-2 value is empirically set at 4 mg/kg-min. For the 20 min and subsequent samples, the computed SM1-2 values become available and are used. The FMi-1 value for the 10 min blood glucose computation is assumed to be 1.0; after that time computed FMi-1 is used.

STATISTICAL ANALYSIS

The biologic activity of the insulins was evaluated by calculating the amount of glucose required to maintain euglycemia during the insulin infusions. It was calculated either as a total amount needed during 6–h infusion, or as the amounts infused during each 2–h insulin infusion. Data was analyzed by using SPSS ver-11. Results were presented by Mean ± Standard deviation. ANOVA test was applied for comparison of three paired group observations at $p < 0.05$.

RESULTS

In 30 cases 24 $\pm$ 3 minutes elapsed before the blood glucose fell by 5 mg/dl to the desired clamp level of blood glucose, suggesting no difference in rate of onset of

action. During the experiment the maximum fluctuation in the blood glucose was 15 mg/dl. Expressed as percentage, blood glucose was 89% of pre-insulin infusion glucose for actrapid insulin, 88% for humulin R and 90 %for zansulin R during all infusion levels.

The overall stability of blood glucose concentration and similar serum glucose values achieved with the three different brands of insulin (figure).

The mean glucose consumed with 0.02 units/kg/hr of Zansulin, Actrapid and Humulin were 26.7gm, 30.6gm and 31.2gm respectively. Similarly the mean glucose consumed with 0.032 units/kg/hr of Zansulin, Actrapid and Humulin were 53gm, 59.7gm and 62.1gm respectively and a similar pattern of glucose consumption was observed for insulin dose of 0.05 units/kg/hr (table-1). As expected there was an increase in glucose requirement with an increasing insulin dose.

The p-value was < 0.05 for three brands of insulin at all three infusion rates. Total glucose administered during the 6 hour infusion also differed significantly among the three brands of insulin, p <0.05 (table-1). Post hoc test for multiple comparisons using Tukey HSD was applied to calculate the significance among three groups. There was significantly more glucose consumption with Humulin R as compared to Zansulin R in all three dosage ranges (p value < 0.05). Similarly glucose consumption was significantly more with Actrapid as compared to Zansulin R (p-value < 0.05). There was no significant difference in biological activity of Humulin R and Actrapid in three dosage ranges (p-value > 0.05) (table-2).

Table-1: Glucose requirement to maintain euglycemia du human insulin the values are presented as g/2h, total amo

Insulin Infusion Units/kg/h	Actrapid	
0.02	30.6 ± 4.5	
0.032	59.7 ± 6.3	
0.05	86.7 ± 7	
Total glucose infused (g)	177 ± 15.5	

(Given data is mean ± standard deviation of the findings) Signific A repeated measure ANOVA was applied for measuring differenc

DISCUSSION

The results showed that using the glucose clamp technique, the three brands of insulin have significantly different hypoglycemic effects at three different insulin dosages. The low insulin infusion rate was chosen to

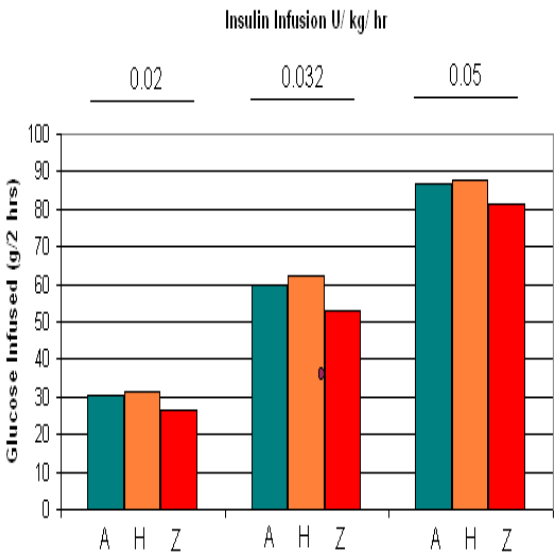

Figure: Glucose infused to maintain euglycemia during infusion of three brands of BHI. Data is expressed as mean of glucose infused over 6 hr for the three insulin doses. A: actrapid, H: humulin R, Z: zansulin R.

Table-2: Post Hoc analyses of multiple comparisons by using Tukey Honestly Significant Difference (HSD).

Insulin Infusion Units/Kg/hr	Insulin brand	Insulin brand	P-value*
0.02	Zansulin R	Actrapid	0.006
		Humulin R	0.001
	Actrapid	Zansulin R	0.006
		Humulin R	0.859
0.032	Zansulin R	Actrapid	0.001
		Humulin R	0.000
	Actrapid	Zansulin R	0.001
		Humulin R	0.350
0.05	Zansulin R	Actrapid	0.018
		Humulin R	0.004
	Actrapid	Zansulin R	0.018
		Humulin R	0.877

examine effects of insulin in liver, while the highest dose should have a hepatic as well as a marked exrtrahepatic effect [2]. The data therefore suggest that the three brands of BHI have significantly different hypoglycemic effects in all dosage ranges.

The use of glucose clamp technique has major advantage over other methods of testing the potency of insulin in human beings. The method employed by Keen et al [3] in their publication was to give insulin subcutaneously or intravenously and then document the rate of fall of blood glucose concentration and the absolute level of glucose attained [3]. Interpretation of such experiments was complicated by the counter regulatory response to a falling glucose concentration involving the secretion of glucagon, cortisol, growth hormone, and catecholamine [4]. This response would vary from individual to individual and would also vary with the rate of fall of glucose. Thus by this technique only a rough estimate of the potency of insulin could be obtained. For more accurate estimations glucose clamp technique remains the preferred method. In glucose clamp technique, euglycemia is maintained and counter regulatory responses are eliminated. The potency of the insulin is assessed by the amount of glucose that has to be infused to maintain the desired glucose value. In our study we chose the desired blood glucose 5 mg/dl below the pre-insulin infusion level to assess the rate of onset of action of different BHI. We find no difference in the rate of onset of action of the three types of insulin.

In our experiment blood glucose clamp was maintained by frequent intermittent glucose measurements. This method is accurate but is more complex to perform as compared to the glucose controlled insulin infusion system (Biostator, Life Science Instruments, Munich, Germany) in which continuous blood glucose monitoring is used to control glucose infusion [5]. In this study subjects with any component of the metabolic syndrome, smokers and offspring of the hypertensive patients were excluded. All these conditions are known to produce insulin resistance and could have confounded the results [6-8]. In our study intravenous route of administration was used but we find no reason that the results will be different by other routes of administration. Our study showed significant variation in glucose consumption for the three brands of insulins in all the three dosages. This study is unique in that it can be reproduced in the office of any physician.

CONCLUSION

Different brands of insulins can be compared easily and accurately for their bioequivalence. Humulin R and Actrapid consumed significantly more glucose in all three dosages as compared to Zansulin R. Humulin R and Actrapid were bioequivalent. Similar models need to be developed for comparing other commonly used drugs.

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