A Model for Evaluation of the Peroral Insulin Therapy: Short-Term Treatment of Alloxan Diabetic Rats with Oral Water-in-Oil-in-Water Insulin Emulsions

MOTOAKI SHICHIRI, RYUZO KAWAMORI, YOSHIKAZU GORIYA, NOBUYOSHI OJI*, YUKIO SHIGETA AND HIROSHI ABE

First Department of Medicine, Osaka University Medical School, Fukushima-ku, Osaka 553, Japan

Synopsis

Alloxan diabetic rats with fasting blood glucose levels above 300 mg/100 ml were treated with oral administration of water-in-oil-in-water (W/O/W) insulin emulsions at a dose of 50 U/100 g body weight, three times daily for 10 to 14 days. The course of diabetes was followed by determinations of glucose levels in blood and urine.

During treatment with oral W/O/W insulin emulsions, daily excretion of urinary glucose decreased by about 30 to 40% (2 to 3 g/day) in all of the five rats studied, and returned to the pre-treatment levels after the treatment being discontinued. During treatment, a significant reduction in fasting blood glucose levels was observed in 4 out of 5 rats, giving the decrease by 18 to 44%. Quantitative estimates suggested that the effectiveness of 50 U/100 g of oral W/O/W insulin emulsions was comparable to that after intramuscular regular insulin at a dose of 0.5 U/100 g.

Although oral W/O/W insulin emulsions are still of low efficiency, these results would indicate that diabetes can be controlled by effective oral insulin preparations.

Although the earlier reports by Lasch and Schönbrunner (1938), Lasch (1951) and Murlin et al. (1940) were somewhat encouraging to the hope of adequate control of diabetes mellitus with oral insulin, attempts to demonstrate any kind of insulin preparation for oral administration have been largely unsuccessful (Danforth and Moore, 1959; Galloway and Root, 1972; Shichiri et al., 1971; Shigeta et al., 1972).

Recent studies in our laboratory have suggested that water-in-oil-in-water (W/O/W) insulin emulsions, when given enterally, exert insulin action by facilitating gastrointestinal absorption and by protecting the insulin molecule from digestive destruction (Shichiri et al., 1974). The question which may now be asked is whether diabetes can be controlled by orally administered insulin preparations. To answer this question, we have studied the short-term treatment of alloxan diabetic rats with daily oral administration of W/O/W insulin emulsions.

Materials and Methods

Preparation of water-in-oil-in-water (W/O/W) insulin emulsions

A method for preparing W/O/W insulin emulsions was described in detail in the previous report (Shichiri et al., 1974). Twelve milliliters of 0.03 M palmitic acid in octyl-decyl triglyceride (Nisshin Seiyu Co., Japan) was placed in a beaker, and then sonication was begun and 8 ml of insulin solution at a concentration of 1,000 U/ml (26.4 U/mg, bovine crystalline insulin, Sigma Chem. Co.) was allowed to drain from a pipette into the beaker. Sonication was continued for about 20 sec with a sonifier (Model USV-3000 V, 22 KHz). This resulted in a water-in-oil (W/O) in-
sulin emulsions. Twenty milliliter of the resulting W/O emulsions was added to a second aqueous phase (60 ml) containing 1% sodium lauryl sulfate (Nikko Chem. Co., Japan). Re-sonication was carried out for 20 sec. The resulting emulsions (W/O/W insulin emulsions) were adjusted to pH 6.5 with dilute NaOH and stored at 4°C for nearly one month. The W/O/W insulin emulsions thus prepared contained insulin at a concentration of 100 U/ml emulsions. Insulin free emulsions were prepared as a control.

Experimental procedures

Male albino rats, Wistar strain, weighing from 200 to 250 g were used. The rats were deprived of food for twenty-four hr and alloxan was injected intravenously at a dose of 4.5 mg/100 g. One month after alloxan injection, fasting blood glucose values were measured for five to seven consecutive days. The rats were randomly used if the mean pre-treatment blood glucose was greater than 300 mg/100 ml. W/O/W insulin emulsions at a dose of 50 U/100 g were given through a gastric tube into the stomach three times daily 30 min before feeding for as long as 14 days. In pre- and post-treatment periods, the same amounts of W/O/W emulsions without insulin were given as controls. During the experimental periods, each rat was maintained on constant food intake three times daily (9 a.m., 1 p.m. and 5 p.m.). The total amounts varied from 15 to 30 g of lab chow per day (Oriental Co., Japan, 1 g = 3.7 Cal.), depending on the ability of food intake in each rat. The rats had free access to water.

The course of diabetes was followed by determinations of fasting blood glucose level and the amount of urinary glucose excreted for 24 hr, and change in body weight. In a few rats, diurnal changes in blood glucose were also studied by obtaining blood samples as indicated in Figure 1. In an attempt to quantitate the effectiveness of oral W/O/W insulin emulsions, changes in the amounts of urinary glucose excreted were compared with those after intramuscular injection of regular insulin. Blood and urinary glucose were determined by the method of Somogyi-Nelson (Nelson, 1944).

Results

Diurnal change in blood glucose

Diurnal changes in blood glucose in alloxan diabetic rats treated with oral administration of W/O/W insulin emulsions are shown in Fig. 1. The experiments consisted of alternative successive days, one on W/O/W emulsions and the next on 50 U/100 g of W/O/W insulin emulsions, with all other conditions identical. After oral administration of W/O/W insulin emulsions 30 minutes before feeding, the degree of postprandial hyperglycemia was reduced with concomitant decrease in the amount of urinary glucose. Fasting blood glucose levels following W/O/W insulin emulsions tended to decrease in both rats.

Short-term treatment of alloxan diabetic rats

Daily oral administration of W/O/W insulin emulsions was studied for as long as 14 days in 5 diabetic rats. The results are shown in Figs. 2 and 3, and are summarized in Table 1.

During treatment, all diabetic rats studied showed a significant reduction in the

![Fig. 1. Diurnal changes in blood glucose in alloxan diabetic rats treated with oral administration of W/O/W insulin emulsions. W/O/W insulin emulsions at a dose of 50 U/100 g are given through a gastric tube into the stomach (--). W/O/W emulsions without insulin are given as controls (- - - - - -). During the experimental periods, the food intake is kept constant at the same calories.](image-url)
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ORAL INSULIN TREATMENT IN DIABETIC RATS

Fig. 2. Short-term treatment of an alloxan diabetic rat (No. 41) with oral W/O/W insulin emulsions or intramuscular injection of regular insulin. W/O/W insulin emulsions at a dose of 50 U/100 g are given orally three times daily for 14 days. Regular insulin at a dose of 2 U/100 g is injected intramuscularly three times daily for 7 days.

Table 1. Effects of short-term treatment of alloxan diabetic rats with either oral administration of W/O/W insulin emulsions or intramuscular injection of regular insulin on fasting blood glucose and the amounts of urinary glucose excreted

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Treatment</th>
<th>Blood Glucose (mg/100 ml)</th>
<th>Urinary Glucose (g/day)</th>
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<tr>
<td></td>
<td></td>
<td>Pre-treatment</td>
<td>Post-treatment</td>
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During the experimental periods, each rat is maintained on constant food intake three times daily (9 a.m., 1 p.m. and 5 p.m.)

Pre- and post-treatment periods consist of 5 to 7 consecutive days.

Values are expressed as mean±SEM.

*: p<0.05, (Treatment) period vs. (Pre-+Post-treatment) period.

**: Change (%)=[(Treatment)/(Pre-treatment+Post-treatment)/2]×100.
amounts of urinary glucose as indicated in figures. When W/O/W insulin emulsions were discontinued, urinary glucose again returned to the pre-treatment levels. The effects of treatment on fasting glucose levels, on the other hand, were quite variable, as observed in the figures. A very good effect on fasting blood glucose levels was observed in rat No. 41 (Fig. 2). During treatment, rat Nos. 48 (Fig. 3), 49 and 50 had a slight but significant decrease in fasting blood glucose levels. In these rats, when W/O/W insulin emulsions were stopped, the fasting blood glucose levels subsequently increased to the pre-treatment levels. In rats No. 47, W/O/W insulin emulsions failed to produce a significant change in blood glucose levels despite the reduction in the amounts of urinary glucose.

To estimate the effectiveness of oral W/O/W insulin emulsions, the results were compared with those after intramuscular injection of regular insulin. In general, as may be seen in Fig. 2, daily intramuscular injection of regular insulin did demonstrate a significant fall in the amounts of urinary glucose excreted, while less change in fasting blood glucose levels. As judged by the changes in the amounts of urinary glucose, the effectiveness of oral insulin was compatible to that after intramuscular insulin at a dose of 0.5 U/100 g (Table 1).

During treatment, the body weight did not change significantly. None of the rats had severe hypoglycemia and gastrointestinal side effects such as diarrhea during treatment.

Discussion

It is interesting to note the possible use of water-in-oil-in-water emulsions as a means of facilitating gastrointestinal absorption of normally non-absorbed water soluble biopolymers (Engel et al., 1968). W/O/W insulin emulsions prepared in our laboratory contained insulin at a concentration of 100 U/ml, with the diameters of the W/O droplets in water in the range of 0.6 to 2.0 μm (Shichiri et al., 1974). Since W/O/W insulin emulsions are considered to exert insulin action by facilitating gastrointestinal absorption and by protecting insulin molecule, to some extent, from digestive degradations, when given intra-jejunally or orally to normal rabbits (Shichiri et al., 1974), their potential use as an oral insulin preparation has been examined in the present experiments.

In a preliminary experiment, we demonstrated that rats required 5 to 6 times more insulin per weight to demonstrate the same effect as observed in rabbits. With a single oral administration of W/O/W insulin emulsions at doses of 40 to 50 U/100 g to alloxan diabetic rats, the blood glucose decreased by about 100 to 150 mg/100 ml during the four-hour experimental period.

Since oral insulin works best on an empty stomach, and might afford a greater chance of absorption, the “three times feeding” with oral W/O/W insulin emulsions 30 min before each feeding would

Fig. 3. Short-term treatment of an alloxan diabetic rat (No. 48) with oral W/O/W insulin emulsions. W/O/W insulin emulsions at a dose of 50 U/100 g are given orally three times daily for 14 days.
give better results to the short-term treatment of alloxan diabetic rats. It is obvious from the results that oral W/O/W insulin emulsions given in the manner described had a significant effect on the excretion of urinary glucose in all of the five rats. During treatment for 10 to 14 days, daily excretion of urinary glucose decreased by 2 to 3 g/day, and returned to the pretreatment levels after the treatment was discontinued, indicating that improvement was not due to a spontaneous remission.

During treatment with oral W/O/W insulin emulsions, a significant reduction in fasting blood glucose levels was observed in 4 out of 5 rats, giving the decrease by 18 to 44%. Intramuscular injection of regular insulin three times daily, on the other hand, failed to show a significant decrease in fasting blood glucose levels, despite of a marked reduction of urinary glucose levels. The different responses of fasting blood glucose levels to oral W/O/W insulin emulsions and intramuscular regular insulin might account for the time-difference of insulin absorption. Plasma insulin reached a peak 15 to 30 min after intramuscular injection of regular insulin. W/O/W insulin emulsions, on the other hand, were absorbed from the intestine, giving the peak absorption at 30 to 60 min when given into the empty stomach (Shichiri et al., 1974). Since W/O/W insulin emulsions are not appreciably absorbed from the stomach, insulin might be absorbed gradually after reaching the jejunum when given orally. W/O/W insulin emulsions mixed with dietary residues might go slowly into the jejunum. As observed in the diurnal changes in blood glucose, postprandial hyperglycemia was reduced after each oral W/O/W insulin emulsion, and then blood glucose gradually increased to the level of fasting blood glucose below that of the day before.

It was hoped that the fasting blood glucose levels might also be made the basis of estimation of the effectiveness, but this appeared to be disappointing on account of the variability in the behavior of the fasting blood glucose levels. When judged by the changes in urinary glucose levels, therefore, the effectiveness of oral insulin was compatible to that after intramuscular insulin at a dose of 0.5 U/100 g.

In the present experiments, we have used very severe diabetic rats, since spontaneous remission of diabetic state is often observed in mild diabetic rats. The present experiment, however, indicate that diabetes could be controlled by oral administration of insulin preparations. Further studies to improve the efficiency of the preparation are necessary for developing effective oral insulin preparations.

Acknowledgement

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References