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THE SYNERGISTIC EFFECTS OF ULTRASOUND AND LASER ON THE ENHANCEMENT OF TRANSDERMAL INSULIN DELIVERY IN DIABETIC RATS

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Abstract

The cardinal goal of this study was to examine the effect of 3-MHz ultrasound and/or laser on transdermal insulin delivery and glucose level in diabetic rats. Diabetes was induced in 45 male rats using Streptozotocin. Animals were randomly divided into 9 groups of 5 animals each. A leak-proof chamber containing either normal saline or insulin was placed on the shaved abdominal skin of the rats. These groups include; rats with no intervention (G1); those treated only with normal-saline (G2); those treated only with insulin (G3); those exposed to laser with normal-saline in the chamber (G4); those exposed to laser with insulin in the chamber (G5); those exposed to ultrasound with normal-saline in the chamber (G6); those exposed to ultrasound with insulin in the chamber (G7); those exposed to both laser and ultrasound with the chamber filled with normal saline (G8) and finally those exposed to both laser and ultrasound with the chamber filled with insulin (G9). The level of blood glucose was measured at minutes 0, 15, 30, 45, 60, 75, 90, 105, and 120 after starting the experiments. The minute 0 refers to the exact time that the anesthetic was administered (before starting irradiation with either laser or ultrasound). Non-parametric tests including Wilcoxon, Kruskal –Wallis, and Mann-Whitney were used for data analysis. These results clearly indicate that the glucose level in rats exposed to both irradiations (laser plus ultrasound) was significantly less than those of exposed to any of these two irradiations alone. To

the best of our knowledge, this study is the first investigation that assesses the combination effect of these two physical interventions in physical agents- mediated drug delivery. These findings may open new horizons in non-invasive drug delivery.

Key terms: Diabetes, Transdermal drug delivery, Insulin, ultrasound, laser.

Introduction

Diabetes is an essential health problem in most societies. In type I diabetes there is a requirement for daily injection of insulin in various doses (1). Utilizing needles for multiple injection of insulin for controlling diabetic patients can be unpleasant. Several noninvasive methods exist for transdermal insulin delivery including sonophoresis and pressure wave (2, 3). Recent studies indicate that ultrasound and pressure wave mediated transdermal insulin delivery offer promising potential for noninvasive drug administration (4-8). Ultrasound is a sound with frequency higher than 20 kHz (9). There are three sets of the ultrasound based on frequency range:

- High-frequency (3-10 MHz)
- Medium-frequency (0.7-3 MHz)
- Low-frequency (18-100 kHz) (9).

Ultrasound applied at both Medium-frequencies and low-frequencies have been used to enhanced transdermal drug delivery(10, 11). Pressure waves, which are generated by intense laser radiation, can be used for transdermal insulin delivery. The application of pressure waves does not cause any pain or discomfort for patient. It must be noted that the interactions of laser with living tissues are entirely different from those induced by ultrasound (7). We know, the synergistic effects of ultrasound and a wide variety of chemical and physical agents (including chemical substances, Iontophoresis and electroporation) have been studied so far but our study that was focused on the possible synergistic effect of ultrasound and pressure waves is the first study in this field (12-16).

1. Method

1.1. Sampling

The rats used in this study were 45 male ones (Sprague Dawly), they kept in special cages in standard conditions for one week. To be adapted that to the new conditions. Their weights were about 200 to 250 gr. These rats categorized into 9 groups, each of which contained 5 ones after becoming hyperglycemic.

These groups include: rats with no intervention (G1); those treated only with normal-saline (G2); those treated only with insulin (G3); those exposed to laser with normal-saline in the chamber (G4); those exposed to laser with insulin in the chamber (G5); those exposed to ultrasound with normal-saline in the chamber (G6); those exposed to ultrasound with insulin in the chamber (G7); those exposed to both laser and ultrasound with the chamber filled with normal saline (G8) and finally those exposed to both laser and ultrasound with the chamber filled with insulin (G9). . Summary of groups showed in table one.

Table-1: Experimental Conditions

Group No.	Experiment Condition
G 1	Diabetics
G 2	Diabetics + Normal-Saline
G 3	Diabetics + Insulin
G 4	Diabetics + Laser + Normal-Saline
G 5	Diabetics + Laser + Insulin
G 6	Diabetics + Ultrasound + Normal-Saline
G 7	Diabetics + Ultrasound + Insulin
G 8	Diabetics + Ultrasound + Laser + Normal-Saline
G 9	Diabetics + Ultrasound + Laser + Insulin

The results of Kruskal-Wallis showed that there were no significant differences at zero time among all groups with respect to glucose level ($p= 0.179$), meaning that at the very beginning of this study all groups were at the same level of glucose.

1.2. Materials

The materials employed in this study consist of Streptozotocin (STZ), Ketamine Hydrochloride, Xylazine, Glucometer, and Human Regular Insulin. Moreover, laser-therapy (LASER 755 - EMS Physio Ltd.) and ultrasound-therapy (ULTRASOUND 215X - Novinmed) were used to do the experiment.

1.3. Procedure

We used a single injection (60 mg/kg) of STZ for induced diabetes in rats in 24-48 hours. Animals with blood glucose level over 250 mg/dl were defined as STZ induced diabetic rats (17-19).

To do the experiment, the rats were anesthetized by Ketamine and Xylazine, and their abdomen hair was shaved. Then, they were put in the restrainer which was designed for this specific study; the chamber which was placed on the abdomens` rats was filled with 3 ml normal-saline and insulin while they were replenished. After that, the chamber was radiated with laser, ultrasound, or both of them consecutively. The features of the laser and ultrasound are given in the following table.

Table-2: The features of laser and ultrasound.

	Ultrasound	Laser
Frequency	3 MHz	500 Hz
Energy	----------	6 joules
Duty cycle	20%	9%
Intensity	$2\ ^{W}/_{cm^2}$	----------
Output power	----------	100 mW
Wavelength	----------	905 nm
Pulse duration	----------	200 ns

This point needs to be mentioned that the laser used was divergent (Rectangular) and its ray was invisible. To resolve the invisibility problem, the IR-detector was placed on the insulin chamber and it was divided into four parts, and as a result for four minutes was devoted to end treatment. In the following table the duration of radiation in all groups is given in minutes.

Table-3: Duration of radiation in all groups.

Group No.	Ultrasound (min)	Laser (min)
G 1	0	0
G 2	0	0
G 3	0	0
G 4	0	1
G 5	0	1
G 6	5	0
G 7	5	0
G 8	5	1
G 9	5	1

And their blood glucose concentrations were measured at 0, 15, 30, 45, 60, 75, 90, 105, and 120 minutes after beginning the experiments. The time 0 minute refers to the exact time after anesthetic was administered and the time before the start of radiation.

To analyze the data, SPSS (Version 19) was utilized and Wilcoxon, Kruskal –Wallis, and Mann-Whitney tests were applied to the data.

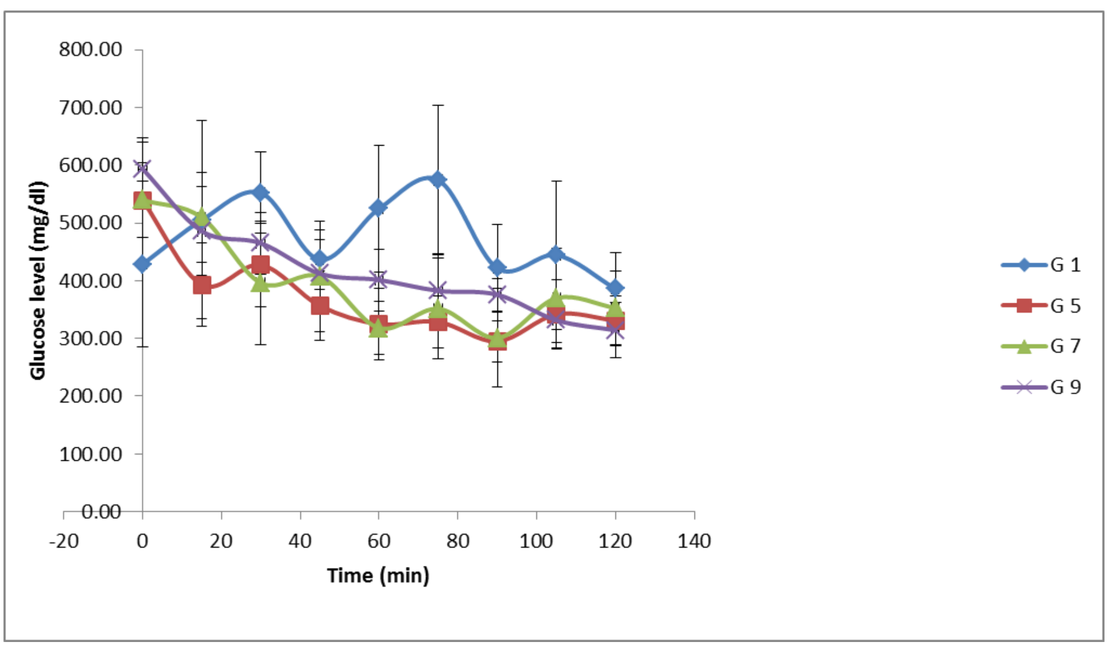
2. Results

The mean scores of blood glucose level at 120 minutes has decreased from 594(SD=48) to 314 (SD=47) in group 9. Moreover, the mean scores of blood glucose level has decreased from 540 (SD=107) to 353 (SD=63) in group 7 at 90 minutes and In group 5, the mean scores of blood glucose level has also decreased from 539 (SD=64) to 301 (SD=43) at the same time. And the blood glucose level in all other groups did not decrease noticeably, as shown in table 4. Results of mean glucose level in control group (G1) Vs. Treatment groups (G 5, G 7, and G 9) were shown in figure 1.

Table-4: The blood glucose level in treatment groups.

Group No.	Initial mean glucose level ± SD (mg/dl)	Mean glucose level. ± SD (mg/dl)
G 5	539 ± 64	301 ± 43 (at 90 min)
G 7	540 ± 107	353 ± 63 (at 90 min)
G 9	594 ± 48	314 ± 47 (at 120 min)

Figure-1: Shows the results of mean glucose level in treatment groups.



There were found significant differences among G5 (0.028 = P-value <0.05), G 7 (0.012 = P-value <0.05), and G 9 (0.043 = P-value <0.05).

Table-4: The results Wilcoxon for mean glucose level between 0 and 120 min in treatment groups.

Group NO.	P-value
G 5	0.028 (0 and 90 min)
G 7	0.012 (0 and 90 min)
G 9	0.043 (0 and 120 min)

Examination of the skin by the naked eye after exposure to insulin, ultrasound and laser showed no vestige of burns or erythema. Histological findings also exhibited no sign of inflammation or destruction of tissue.

3. Discussion

As already mentioned, in this study we aimed at examining the effect of 3 MHz ultrasound and laser on Transdermal Insulin Delivery. The results revealed that the diabetic rats taken insulin in chamber and were radiated with ultrasound and laser decreased in their blood glucose level more than other types of treatment groups.

The results reported in this study clearly indicate that the synergistic effect of Ultrasound and laser is effective in decreasing blood glucose levels in hairless diabetic rats more than ultrasound and laser alone.

In this study, we showed that the best reduction in blood glucose levels should be obtained with both ultrasound (5 min) and laser (1 min) exposure, which is 33% and 15% less than mean blood glucose level for using only US or laser respectively. To proposed method for transdermal insulin delivery especially with replenishment of insulin leads to decrease in side effects such as erythema.

For better clarification of synergistic effect of US and laser in transdermal drug delivery, it is necessary to examine not only different times, wave length and power of pressure waves but also wider range of US frequency, duty cycle, and intensity. Using new multi-modality noninvasive drug delivery methods could make better life for patients suffering from chronic diseases such as diabetes mellitus.

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