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A lecithin-based microemulsion of rh-insulin with aprotinin for oral administration: Investigation of hypoglycemic effects in non-diabetic and STZ-induced diabetic rats

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Abstract

The aim of this study was to develop a microemulsion formulation providing an improved efficacy of orally administered insulin. The microemulsions were prepared using Labrafil M 1944 CS, Phospholipon 90 G (lecithin), absolute alcohol and bi-distilled water. The microemulsions of recombinant human (rh)-insulin and aqueous solution (200 IU/kg) were administered intragastrically by a canulla to diabetic and non-diabetic rats. Aprotinin (2500 KIU/g) was added as the enzyme inhibitor to the formulation. Upon the administration of intragastric rh-insulin solution (IS) to non-diabetic rats, the plasma glucose and insulin levels were not changed significantly. Therefore, the hypoglycemic effect caused by subcutaneous rh-insulin solution (SC), microemulsion containing rh-insulin (IME) and microemulsion containing rh-insulin and aprotinin (IMEA) were analyzed in diabetic rats. The area above the plasma glucose levels time curves (AAC), minimum glucose concentration (C_{min}) and time to C_{min} (t_{min}) were derived from the plasma glucose profiles. IME and IMEA caused approximately 30% decrease in plasma glucose levels. The decrease in the plasma glucose levels continued after the 90th min. The highest AAC value was obtained when IMEA was administered to rats. The maximum plasma insulin concentration (C_{max}), time to reach C_{max} (t_{max}), terminal half-life ($t_{1/2}$), area under the plasma concentration–time curve (AUC), mean residence time (MRT) and elimination rate constant (k_{el}) values were also calculated. It was observed that $t_{1/2}$ values varied between 0.53 and 1.31 h. No significant difference could be found between the pharmacokinetic parameters of the IME and IMEA administered groups. Addition of aprotinin to the microemulsion containing rh-insulin increased bioavailability when compared to those not containing it, although the difference is not significant.

Keywords: Insulin; Microemulsion; Oral administration; Lecithin; Hypoglycemic effect

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1. Introduction

Since the discovery of insulin, this 51 amino acid peptide has generally been administered parenterally in the treatment of diabetes mellitus (Beals and Kovach, 1997). Unfortunately, injections are often painful, and can lead to low patient compliance. The delivery of insulin by the non-parenteral routes has gained significant attention over the last two decades. Oral delivery of drugs, especially therapeutic proteins, is the preferred route of administration because it offers advantages over injections, which is the accepted route of therapeutic protein administration. We believe that oral delivery is an improved method of insulin delivery, much easier than dealing with injections, and that it can lead to improved patient compliance. There are several limitations to the oral route of insulin delivery, including low oral bioavailability due to degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity (Carino and Mathiowitz, 1999; Lee and Yamamoto, 1990). Therefore, various approaches have been examined to overcome the delivery problems of these peptides when they are administered orally (Wang, 1996; Kompella and Lee, 2001). Of these, the use of carrier systems, such as liposomes (Kisel et al., 2001), mixed micelles (Scott-Moncrieff et al., 1994), multiple emulsions (Silva-Cunha et al., 1998), microemulsions (Çelebi et al., 2002), microspheres (Kim et al., 2002), hydrogels (Kim and Peppas, 2003), azopolymer coating (Saffran et al., 1991), site-specific drug delivery systems (Tozaki et al., 1997), absorption enhancers (Ziv et al., 1987), enzyme inhibitors (Yamamoto et al., 1994) and modification of chemical structure (Asada et al., 1995) has been shown to improve the gastrointestinal absorption of peptide drugs. Among these systems, the microemulsion seems to be a suitable carrier for orally administered peptide drugs.

Microemulsion systems have received increasing attention during the past few years. Formulations based on microemulsions have several advantages over conventional formulations, namely thermodynamic stability, enhanced drug solubilization and ease of manufacturing (Lawrence and Rees, 2000). Lipid-based microemulsions (o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides. This delivery system may protect proteins from proteolysis or acidic degradation, and thus enhance the protein absorption in the gastrointestinal tract. Hydrophilic drugs of this kind can be successfully incorporated into the dispersed aqueous phase of w/o microemulsion droplets where they are afforded some protection from enzymatic degradation when administered orally (Sarciaux et al., 1995; Ritschel, 1991). Sandimmune Neoral®, an example of a marketed microemulsion formulation, is a microemulsion preconcentrate containing a surfactant, lipophilic and hydrophilic solvents and ethanol (Tenjarla, 1999). In addition, the presence of surfactant and in some case co-surfactant, for example, and medium chain diglycerides in many cases, serves to increase the membrane permeability, thereby increasing drug uptake (Constantinides et al., 1994).

Recently, considerable dosage form development activity has focused on the formulation of lecithin-based microemulsions (Paolino et al., 2002; Trotta et al., 1998; Park et al., 1999). Lecithin is a naturally occurring, non-toxic and safe material. It is also a biological surfactant and a major component of membrane lipids.

In light of the above, the objectives of this study were (a) to develop a stable microemulsion formulation of rh-insulin for oral administration and (b) to examine the effects of intragastric administration of rh-insulin microemulsion on plasma glucose and insulin levels and to compare with intragastric aqueous rh-insulin solution and subcutaneous administration in diabetic and non-diabetic rats. In addition, the effect of aprotinin, an enzyme inhibitor, was also investigated.

2. Materials and methods

2.1. Materials

Recombinant human (rh)-insulin (Humulin R 100 IU/mL) was kindly contributed by Lilly Comp. (Turkey), Labrafil M 1944 CS (unsaturated polyglycolysed glycerides) by Gattefosse (France), Phospholipon 90 G by Rhone-Poulenc, Nattermann Phospholipid GMBH (Germany) and aprotinin (Trasylol 6128 KIU/mg) by Bayer Türk (Turkey), streptozotocin (STZ) was purchased from Sigma Chemical Co. (USA) and absolute alcohol was purchased from Riedel de Haen (Seelze, Germany).

2.2. Preparation of microemulsion

The microemulsions were prepared using Labrafil M 1944 CS (oil phase), lecithin (surfactant), absolute alcohol (co-surfactant) and bi-distilled water (aqueous phase). Pseudo-ternary phase diagram was constructed by titration of bi-distilled water into a mixture of oil/surfactant/co-surfactant. The experiments were done at 25 ± 0.5 °C (constant temperature). The mixture of phospholipid (2.04 g) and co-surfactant (2.04 g) was mixed with the 3.76 g of oil phase and the obtained mixture was slowly titrated with 2.16 g of bi-distilled water. In in vivo studies, rh-insulin solution (21.6 IU/g) or the mixture of rh-insulin solution (21.6 IU/g) and aprotinin (2500 KIU/g) was used as the aqueous phase. The type of the microemulsion was found to be w/o as determined by the dye method. The characterization of microemulsion results indicated that the physical characteristics of the developed microemulsion did not change under different storage temperatures (4, 25 and 40 °C) ($p > 0.05$) (Çilek et al., in press). The microemulsions existence field for s/co-s of one is shown in Fig. 1.

2.3. Animals

Male Wistar rats weighing approximately 170–300 g were used throughout the study. The animals were fasted overnight but had free access to water. The animals were maintained at a constant temperature (22 °C) with a fixed 12 h light:12 h dark cycle (lights on 07:00–19:00). They were divided into eight major groups, including 7–10 rats. The microemulsions and rh-insulin solution (200 IU/kg) were administered intragastrically by a canulla, rh-insulin solution (0.3 IU/kg) was administered subcutaneously. The SC administered insulin dose used in studies reported in literature was between 0.25 and 3.0 IU/kg (Morishita et al., 1998). The results of our preliminary studies indicate that a subcutaneous insulin dose of 0.3 IU/kg was suitable. The Ethics Committee of Ankara University reviewed and approved the trial protocol. The design of the experimental animal groups is shown in Table 1.

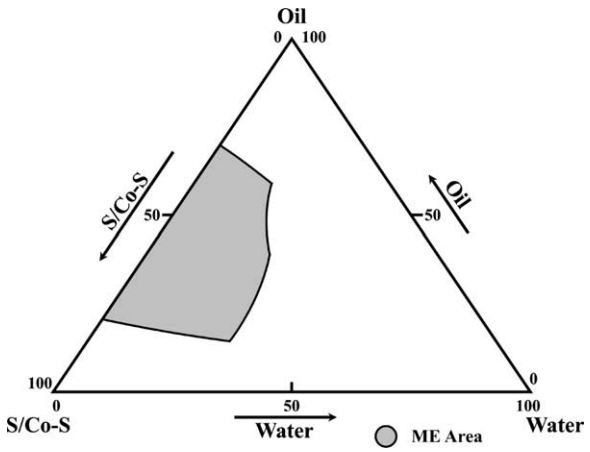

Fig. 1. The pseudo-ternary phase diagram for the microemulsion formulation.

Table 1
Design of experimental animal groups

Code	Administrations
ME	Microemulsion without rh-insulin (non-diabetic rats)
IME-N[a]	Microemulsion containing rh-insulin (non-diabetic rats)
IMEA-N[a]	Microemulsion containing rh-insulin and aprotinin (non-diabetic rats)
IS[a]	rh-Insulin solution (non-diabetic rats)
SC-N[b]	rh-Insulin solution (non-diabetic rats)
IME-D[a]	Microemulsion containing rh-insulin (diabetic rats)
IMEA-D[a]	Microemulsion containing rh-insulin and aprotinin (diabetic rats)
SC-D[b]	rh-Insulin solution (diabetic rats)

[a] Intragastric administration (200 IU/kg).
[b] Subcutaneous administration (0.3 IU/kg).

2.4. Induction of diabetes

Diabetes was induced in male Wistar rats by a single tail vein injection of streptozotocin (45 mg/kg body weight). STZ was dissolved in 1 mL of 0.1 M cold citrate buffer (pH 4.5) immediately before use. One week after the injection of STZ, diabetes was confirmed by measuring glucose concentration in a blood sample obtained from the tail vein. Only rats with fasting blood glucose levels over than 300 mg/dL were considered as diabetics and used in the present study.

2.5. Determination of plasma glucose levels

Blood samples were obtained from tail vein before and 15, 30, 60, 90, 120 and 180 min after administration. The blood samples were centrifuged and plasma glucose levels were determined immediately.

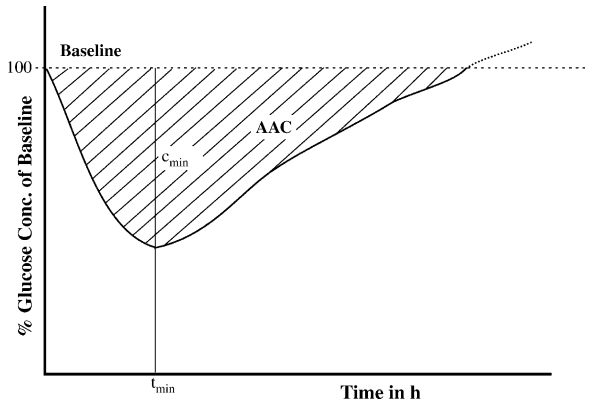

Fig. 2. Schematic diagram for evaluation of glucose concentration–time data.

The plasma glucose levels were determined by the glucose oxydase method (Glucose liquicolor, HUMAN, Wiesbaden, Germany). According to this method, the plasma (10 μL) was added to 1 mL glucose reagent, the tube contents were mixed and were incubated for 5 min at 37 °C. The absorbance values of the standard and plasma samples were measured within 60 min against the reagent blank at 500 nm using the spectrophotometer. The plasma glucose concentration was calculated in mg/dL.

The hypoglycemic response to rh-insulin was characterized as follows: plasma glucose levels after insulin administrations were expressed as a percentage of the initial level. The data evaluation is shown in the schematic diagram Fig. 2. The areas above the plasma glucose levels time curves (AAC) were calculated using the trapezoidal rule (Ritschel et al., 1988).

2.6. Determination of plasma insulin levels

Blood samples were obtained from tail vein at 0, 15, 30, 60, 90, 120 and 180 min. The blood samples were centrifuged and the plasma was stored at −80 °C until the insulin determination. The plasma insulin concentration was determined by radioimmunoassay (RIA kit-Coat-a-Count, DPC, Los Angeles, CA).

2.7. Data analysis

The one-way analysis of variance (ANOVA) followed by Student Newman–Keuls multiple comparisons was used for analysis of the data. Results are expressed as the mean ± confidence interval (CI). $p < 0.05$ was considered as significant for all comparisons.

The relative pharmacological bioavailability (PA%) was calculated using the following equations. The same equation was used in calculation of the relative bioavailability (F%); however, the AUC values were substituted instead of the AAC values (Nakamura et al., 2004).

$$\mathrm{PA\%} = \left(\frac{\mathrm{AAC_{oral}}}{\mathrm{AAC_{SC}}}\right) \times \left(\frac{\mathrm{Dose_{SC}}}{\mathrm{Dose_{oral}}}\right) \times 100$$

$$\mathrm{PA\%} = \left(\frac{\mathrm{AAC_{IMEA}}}{\mathrm{AAC_{IME}}}\right) \times \left(\frac{\mathrm{Dose_{IME}}}{\mathrm{Dose_{IMEA}}}\right) \times 100$$

3. Results and discussion

3.1. Preparation of microemulsion

Due to the fact that the prepared microemulsion formulation was to be administered intragastrically, the substances to be used in the formulation were carefully selected. The absolute alcohol, which was used as the co-surfactant, was preferred since it is safe (Gursoy and Benita, 2004). As lecithin is a non-toxic and biologically compatible substance, microemulsions containing lecithin are preferred (Tenjarla, 1999). In the preparation of the microemulsion formulation, high-purity phosphatidylcholine (93 ± 3%) containing lecithin (Phospholipon 90 G) was used.

3.2. Plasma glucose levels

The percent of reduction from the initial glucose levels versus time profiles after intragastric and subcutaneous administration of rh-insulin to non-diabetic rats are shown in Fig. 3. The mean plasma glucose baseline value was taken as 100% level and all following concentrations–time data was given accordingly as percentage of the baseline. The percentage of reduction of initial glycemia was used as the parameter to evaluate insulin absorption.

As shown in Fig. 3, administration of intragastric aqueous rh-insulin solution (IS), at a dose of 200 IU/kg, did not change the plasma glucose levels. The results of this study performed on non-diabetic rats had shown that microemulsion without rh-insulin (ME) and intra-

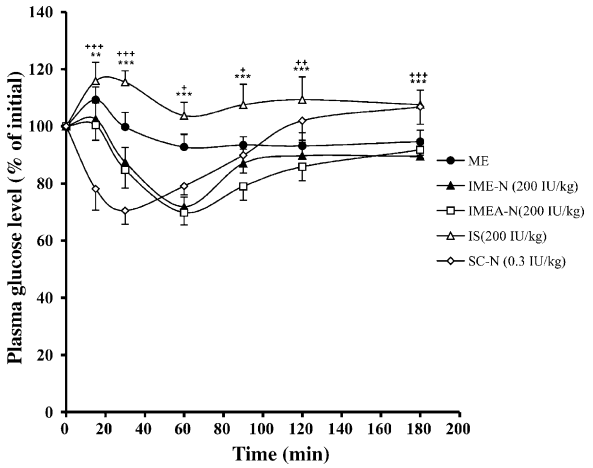

Fig. 3. Percentage reduction in plasma glucose concentration in the non-diabetic rats. Values are expressed as mean ± CI (* and + represents IMEA-N vs. IS and SC-N groups, respectively. $^{+}p < 0.05$; $^{**,++}p < 0.01$; $^{***,+++}p < 0.001$).

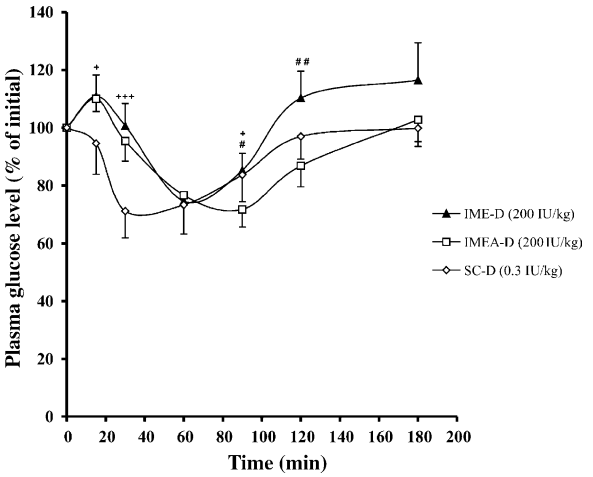

Fig. 4. Percentage reduction in plasma glucose concentration in the diabetic rats. Values are expressed as mean ± CI (# and + represents IMEA-D vs. IME-D and SC-D groups, respectively. $^{+,\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{+++}p < 0.001$).

gastric rh-insulin solutions (IS) do not cause a significant change in the plasma glucose and insulin levels ($p > 0.05$, Wagner, 1979). Therefore, microemulsion containing rh-insulin (IME), microemulsion containing rh-insulin and aprotinin (IMEA) and subcutaneous rh-insulin solution (SC) were administered to the diabetic rats. The percentage of reduction from the initial glucose levels versus time profile, after intragastric and subcutaneous administration of rh-insulin to diabetic rats, are shown in Fig. 4.

In rats administered all intragastric formulations, the plasma glucose levels were higher than the initial levels at the 15th min. (Figs. 3 and 4). This increase is considered to be due to stress provoked by force-feeding and blood sampling under non-anesthetized conditions. As shown in Figs. 3 and 4, it was observed that subcutaneous administration of rh-insulin and intragastric administration of rh-insulin microemulsions in rats reduced the plasma glucose level at almost the same percentage.

A significant difference in plasma glucose reduction (percentage of initial value) between IS and IMEA-N administered groups was observed at all times after the administration ($p < 0.05$). No significant difference in plasma glucose reduction was observed between administration of IME-N and IMEA-N at any of the measured time points ($p > 0.05$). But a significant difference in plasma glucose reduction was observed between 90 and 120 min for IME-D and IMEA-D ($p < 0.05$). In diabetic rats case, IMEA caused more reduction in plasma glucose levels, however, we could not the explain reason.

The effects of aprotinin as enzyme inhibitor on plasma glucose levels are also shown in Figs. 3 and 4. The addition of aprotinin to the formulation provided a reduction in the plasma glucose level up to 30% in our study. It also prolonged the t_{min} of plasma glucose levels in diabetic rats. Although there occurred no significant difference between t_{min} values of IME-N and IMEA-N administered groups ($p > 0.05$), a significant difference was observed between t_{min} values of IME-D and IMEA-D administered groups ($p < 0.001$). In rats the effect of subcutaneous administration reached maximum level at the 30th min. However, with the administration of IME and IMEA the maximum decrease in plasma glucose level was obtained at the 1 and 1.35 h after administration, respectively. In the SC administered group, the reduction in the plasma glucose level ended at the 90th min. On the other hand, in the IME and IMEA administered groups, the decrease in the plasma glucose levels continued after the 90th min. It is normal to have a delay occurring as a result of the absorption region of subcutaneous and intragastric administrations. In SC administration, the rh-insulin passes directly to the blood and causes an immediate effective response. Even in this case the maximum

Table 2
Parameters for plasma glucose levels and relative pharmacological bioavailability (means ± CI, $n = 7–10$)

	IME-N	IMEA-N	SC-N	IME-D	IMEA-D	SC-D
Insulin dose (IU/kg)	200	200	0.3	200	200	0.3
C_{min} (mg/dL)	71.9 ± 3.4	69.8 ± 3.8	67.5 ± 2.5	74.6 ± 3.3	69.6 ± 4.5	69.8 ± 9.4
t_{min} (h)	1 ± 0	1 ± 0	0.55 ± 0.15	1 ± 0	1.35 ± 0.14	0.64 ± 0.18
AAC	2635.5 ± 584.8	2820.3 ± 548.4	2662.8 ± 621.2	2606.6 ± 853.6	3464.1 ± 663.1	2664.9 ± 691.0
PA%[a]	0.148	0.159	100	0.147	0.195	100
PA%[b]	100	107		100	133	

C_{min}, minimum plasma glucose concentration (% of initial); t_{min}, time to C_{min}; AAC, area above the plasma glucose levels time curves; PA%, relative pharmacological bioavailability.
[a] Based on AAC for subcutaneous administration (SC).
[b] Based on AAC for microemulsion containing rh-insulin (IME).

reduction occurs in merely 30 min. The C_{min} value of plasma glucose profile of the IMEA administered group was lower than that of the IME administered group, but the difference being not so significant ($p > 0.05$). The obtained AAC, PA%, C_{min} and t_{min} values are shown in Table 2.

As can be seen in Table 2, the same AAC values were calculated for SC (0.3 IU/kg) and microemulsion administered groups (200 IU/kg) ($p > 0.05$). The highest AAC value was obtained when IMEA was administered to rats, but the differences were not so significant ($p > 0.05$).

Relative pharmacological bioavailability in percentage glucose reduction was calculated as the ratio between the AAC of the IMEA administered group and the AAC of the IME administered group (Table 2). As the PA% values are dependent on the doses, when the SC and insulin containing microemulsions are compared, the bioavailabilities of microemulsions were found to be much lower than that of SC application. The PA% and AAC values of IS and ME groups could not be determined because the decrease in the plasma glucose level were insignificant. But when Figs. 3 and 5 were considered, it can be said that there were significant differences between IME, IMEA and IS, ME administered groups. This is indicated that the microemulsion formulation is capable to protect insulin after oral administration. There are several studies in the literature regarding microemulsions of insulin. In a study done by Kraeling and Ritschel (1992), the peroral microemulsion formulation of insulin and capsule forms were compared. It was observed that the microemulsion increases the bioavailability of the insulin. In a different study, the absorption of the insulin in the gastrointestinal tract with various emulsion systems was investigated ex vivo by Trenktrog et al. (1995). They claimed that microemulsions significantly increased absorption of insulin compared with o/w and w/o emulsions, while insulin solution was not effective. In a study performed by Watnasirichaikul et al. (2002), it was stated that the formulation of insulin within nanocapsules dispersed in the w/o microemulsion had been shown to significantly increase the oral bioavailability of insulin in diabetic rats. In another study, it was stated that the microemulsion formed by polyglycerol fatty acid esthers, cosurfactant and Captex 300, and water is a suitable system for oral delivery of insulin. It was determined that the microemulsion prevents the digestion

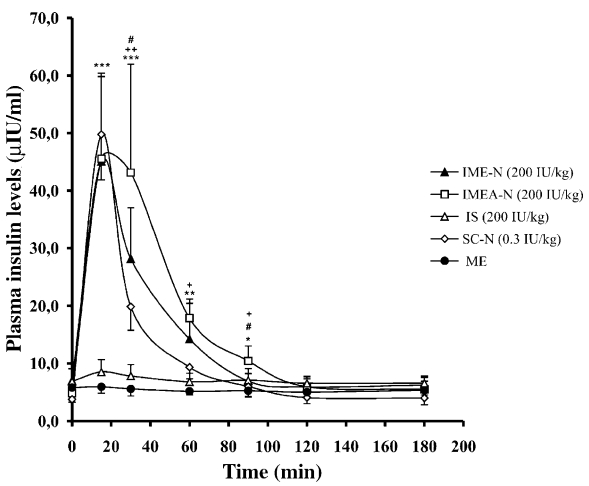

Fig. 5. The plasma insulin levels for non-diabetic rats. Values are expressed as mean ± CI (*, + and # represents IMEA-N vs. IS, SC-N and IME-N groups, respectively. $^{*,+,\#}p < 0.05$; $^{**,++}p < 0.01$; $^{***}p < 0.001$).

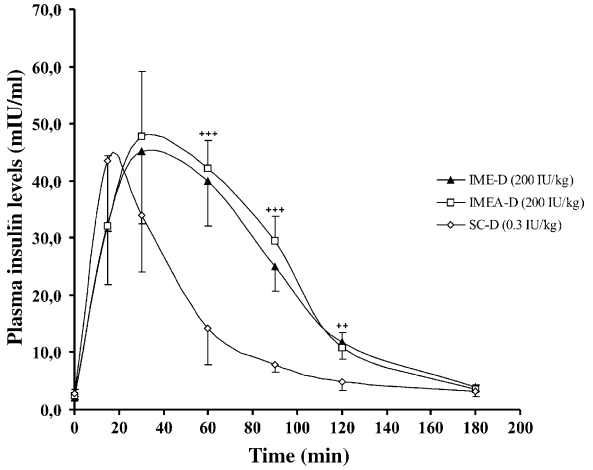

Fig. 6. The plasma insulin levels for diabetic rats. Values are expressed as mean ± CI (+ represents IMEA-D vs. SC-D group. $^{++}p < 0.01$; $^{+++}p < 0.001$).

of insulin by the gastrointestinal tract acid (Ho et al., 1996). Similar results were observed in our study.

3.3. Plasma insulin levels

The mean plasma insulin levels versus time profiles, after intragastric and subcutaneous administration of rh-insulin to non-diabetic rats, are shown in Fig. 5.

Fig. 6 summarizes the changes in plasma insulin levels after administration of different formulations in diabetic rats.

A significant difference in plasma insulin levels was observed between 15 and 90 min for IMEA-N and IS ($p < 0.05$). A significant difference in plasma insulin levels between IME-N and IMEA-N administered groups was observed at 30 and 90 min ($p < 0.05$). No significant difference in plasma insulin levels was observed between administration of IME-D and IMEA-D ($p > 0.05$).

Similar to the case with other endogenous materials, the pharmacokinetics of insulin is difficult to investigate. In our study, basic pharmacokinetics and bioavailability parameters were calculated. As ME and IS did not change the plasma insulin level significantly, these groups were not used in the calculations. The plasma insulin levels caused by SC, IME and IMEA were analyzed independent of the compartment model. For analysis, non-compartmental analysis (NCOMP) program was used. Plasma insulin levels and the time values were inputted in the program. In the calculations, the average plasma concentration values were first plotted on a semi-logarithmic graph. The linear part of the curve was then determined, as it is known to be log-linear phase. Although the total experiment duration was 3 h, the plasma data up to 2 h were used, the average insulin levels were very close at the second and third hour. We observed that the plasma insulin levels did not change after the second hour.

For all groups, when the semi-logarithmic graphs were investigated, it was decided that the last three plasma values were linear. The last three points were inputted in the NCOMP program. The program outputs the C_{max}, t_{max}, k_{el}, $t_{1/2,}$ MRT and AUC values. The results are shown in Table 3.

In some of the analysis, plasma insulin level at the second hour was found to be higher than the previous data. When the obtained plasma insulin levels were inputted to the NCOMP program, negative k_{el} values were obtained, and these values were discarded. As a result, the C_{max} values shown in Table 3 are different from the ones shown in Figs. 5 and 6. No significant difference could be found between the C_{max} values of the IME and IMEA administered groups ($p > 0.05$). When the k_{el} values of diabetic and non-diabetic rats were compared, the values were very close ($p > 0.05$). Naturally, the $t_{1/2}$ values calculated via these values were also close ($p > 0.05$). It was observed that $t_{1/2}$ values varied between 31.8 and 78.6 min. In a study performed by Schilling and Mitra, 1990, $t_{1/2}$ values were found to be between 39.4 and 63.1 min, being independent of the compartment analysis. These values comply with our results. Also, no significant difference was encountered for the t_{max} and MRT values of the all formulations ($p > 0.05$).

The main parameters to be considered in comparison of the formulations are the AUC values that are outputs of the NCOMP program. Using the relative response equality mentioned in the section related to plasma glucose level investigation, relative bioavailability values were calculated. The microemulsions containing aprotinin yielded more bioavailability than those not containing it. But the increase in bioavailability was not significant. When the biological availabilities caused by IME and IMEA were compared, IMEA increased the bioavailability by 15% for the non-diabetic group and 6% for the diabetic group, as can be seen in Table 3. Intragastric delivery of insulin as

Table 3
Pharmacokinetic parameters for plasma insulin levels (means ± CI, $n = 7–10$)

	IME-N	IMEA-N	SC-N	IME-D	IMEA-D	SC-D
Insulin dose (IU/kg)	200	200	0.3	200	200	0.3
C_{max} (μIU/mL)	53.3 ± 15.2	54.0 ± 16.3	49.1 ± 8.8	50.0 ± 9.2	54.8 ± 9.2	44.7 ± 7.6
t_{max} (h)	0.25 ± 0.00	0.36 ± 0.08	0.25 ± 0.00	0.90 ± 0.23	0.83 ± 0.26	0.29 ± 0.07
k_{el} (h)	1.21 ± 0.60	1.13 ± 0.22	0.97 ± 0.19	1.27 ± 0.28	1.42 ± 0.23	1.13 ± 0.47
$t_{1/2}$ (h)	1.31 ± 1.22	0.67 ± 0.13	0.79 ± 0.19	0.62 ± 0.13	0.53 ± 0.09	0.90 ± 0.54
MRT (h)	1.77 ± 1.44	1.05 ± 0.10	1.04 ± 0.14	1.29 ± 0.13	1.21 ± 0.12	1.34 ± 0.63
AUC (μIU h/mL)	34.9 ± 7.2	40.1 ± 7.9	25.5 ± 3.7	57.6 ± 8.9	61.2 ± 6.3	31.5 ± 6.4
F%[a]	0.205	0.236	100	0.274	0.291	100
F%[b]	100	115		100	106	

C_{max}, the maximum plasma insulin concentration; t_{max}, the time to reach the C_{max}; $t_{1/2}$, half-life; MRT, the mean residence time; AUC, the area under the curve; F%, relative bioavailability.

[a] Based on AUC for subcutaneous administration (SC).

[b] Based on AUC for microemulsion containing rh-insulin (IME).

a microemulsion formulation yielded a lower bioavailability when compared to the subcutaneous administration. Furthermore, in the diabetic group, AUC values of IME, IMEA and SC administered groups were found to be higher than those of the non-diabetic group by 65, 53 and 24%, respectively. In conclusion, diabetes of rats caused an apparent increase in AUC level. However, the reason of the high bioavailability in diabetic rats could not be explained.

When administration of IME was compared with IMEA, it was seen that in addition to the insulin-preserving activity of the microemulsion and aprotinin also provided additional preservation effect. Aprotinin has recently been shown to enhance the absorption of a number of polypeptide hormones. It was also observed that aprotinin, which was added as the enzyme inhibitor to the formulation, prevented the insulin from degradation caused by proteolytic enzymes and increased the stability in the intestinal tract (Bernkop-Schnurch, 1998; Nishihata et al., 1983). In the study of Ziv et al. (1987), it was observed that when an insulin solution containing 1000–3000 KIU/mL aprotinin and 10 mg/mL sodium cholate, was applied to rat lumen directly, the absorption increased by 30-fold. Considering the information obtained from the literature, it was decided that it would be suitable to add aprotinin in the amount of 2500 KIU/mL to the prepared microemulsion formulation. In a study performed by Cho and Flynn (1989), a microemulsion formed by lipid phase, containing lecithin, non-esterified fatty acids and cholesterol, and water phase including insulin was prepared and applied to humans. When aprotinin was added to the water phase, an apparent fall in plasma glucose levels and rise in plasma insulin levels were observed. The clinical acceptability of the oral microemulsion formulation of insulin was stated in this study.

4. Conclusion

We attempted to develop a stable microemulsion formulation of rh-insulin for oral administration. The plasma glucose levels and insulin levels of rats were significantly different after oral administration of the microemulsions compared to oral insulin solution. The microemulsion will prevent the enzymatic degradation of rh-insulin in the gastrointestinal system. In conclusion, the employment of microemulsion was found to be a suitable carrier system for the administration of rh-insulin through the oral route. We believe that this study will contribute to the studies on the oral administration of microemulsions that contain peptide drugs.

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insulin, aprotinin, Labrafil M 1994 CS and Phospholipon 90 G, respectively. The authors would like to thank Prof. Dr. A. Tanju Özçelikay for induction of diabetes in rats.

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