FATE OF PORCINE AND HUMAN INSULIN AT THE SUBCUTANEOUS INJECTION SITE. I. DEGRADATION AND ABSORPTION OF INSULINS IN THE RAT

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(Received July 2, 1984)

The plasma insulin and serum glucose levels were compared after the subcutaneous and intravenous administration of porcine and human insulin in rats. While no difference was observed in plasma insulin or serum glucose levels with either insulin after intravenous administration, the plasma insulin levels and hypoglycemic effects of human insulin were greater than those of porcine insulin after subcutaneous administration. At various time intervals, radioactivity in subcutaneous tissue was assayed for insulin and/or its metabolites by gel filtration. Using these data, the absorption and degradation rate constants of these substances were estimated according to a one-compartment model. The degradation rate constants of human insulin was approximately half that of porcine insulin.

Keywords — insulin; porcine insulin; human insulin; plasma immunoreactive insulin; serum glucose measurement; subcutaneous insulin injection; intravenous insulin injection; insulin absorption; insulin degradation

INTRODUCTION

The fate of insulin in the blood after subcutaneous injection is the result of many factors including absorption and degradation in the injection site. Our previous report demonstrated the dynamic nature of the absorption and the degradation of porcine insulin at the subcutaneous injection site.\(^1\) The bioavailability of the porcine insulin injected subcutaneously was lower than expected, and the addition of benzylloxy carbonyl (Z)-Gly-Pro-Leu-Gly remarkable inhibited the degradation.

Recently, human insulin, which differs from porcine insulin by one amino acid of B-chain, has been produced on a commercial scale by two different processes. The first process is an enzymatic conversion to replace the alanine of C-terminal amino acid of B-chain portion of porcine insulin. The second process is recombinant deoxyribonucleic acid (DNA) technology. Several groups have reported that plasma insulin levels and the hypoglycemic effect of human insulin were greater than those of porcine insulin after subcutaneous administration, while no difference was seen after intravenous administration.\(^2\)\(^-\)\(^4\) It should be noted, however, that other reports on subcutaneously injected insulin show no difference.\(^5\)\(^-\)\(^7\) The present studies were undertaken to compare the dynamic nature of the absorption and the degradation of porcine and semisynthetic human insulin at the subcutaneous injection site.

MATERIALS AND METHODS

**Materials** — Monocomponent porcine insulin (26.0 IU/mg) and semisynthetic monocomponent human insulin (26.4 IU/mg) were kindly supplied by NOVO Industri A/S, (Denmark). \(^{[125]}\)I-Porcine and human insulin were prepared by the chloramine-T method as de-
scribed by Hunter and Greenwood.\textsuperscript{8} The labeled insulins were purified by gel filtration twice using Sephadex G-25 and G-50. All chemicals were of analytical reagent grade.

**Procedure** — Male Wistar rats weighing 110–150 g were anesthetized with pentobarbital (40 mg/kg) and maintained under anesthesia during the experiment. The rectal temperature was monitored and maintained constant (36.5 ± 0.5 °C) by keeping the rats in a temperature-constant room. The dorsal skin was depilated and the site for injection was demarcated. Using a microsyringe with a thin needle (O.D. = 0.2 mm and I.D. = 0.1 mm, N-733, Hamilton Co. U.S.A.), 10 or 25 µl of pH 7.0 isotonic phosphate buffer solution containing unlabelled insulin (0.2 U/kg) and \textsuperscript{125I}-insulin was injected subcutaneously in single shot for one rat. The injection site was fixed at same site of dorsal skin. At various times, a skin sample and subcutaneous tissue around the injection site (∼4 cm² area) was taken for analysis. The analytical method was described in our previous report.\textsuperscript{11} For the experiment which was conducted to measure the plasma immunoreactive insulin (IRI) levels\textsuperscript{9} or serum glucose levels\textsuperscript{10} (in 16 h fasted rats), unlabelled porcine or human insulin was injected subcutaneously or intravenously.

**Cardiac Arrested Rats** — To estimate the degradation rate of insulin at the injection site when its absorption would be negligible, the degradation during cardiac arrest in rat was investigated.\textsuperscript{11} This condition was induced by direct injection of the pentobarbital (200 mg/kg) to the heart 5 before the experiment.

**Kinetic Model** — The disposition of insulin in the subcutaneous tissue was analyzed as assuming a one-compartment model as described previously.\textsuperscript{11} Insulin can be biotransformed to low molecular weight product (LMWP, which is monooiodotyrosine) and to high molecular weight product (HMWP, which is mixtures of subcutaneous components bound to undergraded insulin and insulin degradation products), with a degradation rate constant of $K_m$ and formation rate constant of $K_b$, respectively, as shown in Fig. 1. Insulin, LMWP, and HMWP are absorbed independently with absorption rate constants of $K_a$, $K_b$, and $K_c$, respectively. $K_b$ and $K_c$ were determined by the disappearance of subcutaneously injected LMWP and HMWP, respectively in separate experiment. $K_b$ and $K_c$ were fixed in Eqs. 2 and 3. The mean data of each time were fitted to Eqs. 1–3 by nonlinear least-squares regression.\textsuperscript{11}

$X = X_0 e^{-K_t}$ \hspace{1cm} (1)

$L = \frac{X_0 K_m}{K_b} (e^{-K_b t} - e^{-K_t}) + L_0 e^{-K_b t}$ \hspace{1cm} (2)

and

$H = \frac{X_0 K_h}{K_c} (e^{-K_c t} - e^{-K_t}) + H_0 e^{-K_c t}$ \hspace{1cm} (3)

where $X_0$, $L_0$, and $H_0$ are the initial amount of insulin, LMWP, and HMWP, respectively. $K = K_a + K_m + K_b$, and $t$ is the sampling time.\textsuperscript{12}

![Kinetic Model for Insulin at Subcutaneous Injection Site](image)

**FIG. 1. Kinetic Model for Insulin at Subcutaneous Injection Site**

- $K_a$ = absorption rate constant of insulin,
- $K_b$ = absorption rate constant of LMWP,
- $K_c$ = absorption rate constant of HMWP,
- $K_m$ = degradation rate constant of insulin,
- $K_h$ = aggregation rate constant of insulin,
- $X$ = amount of insulin in injection site,
- $L$ = amount of LMWP in injection site,
- $H$ = amount of HMWP in injection site,
- $X_b$ = amount of insulin in body,
- $L_b$ = amount of LMWP in body,
- $H_b$ = amount of HMWP in body.
The degradation rate constant ($K_m$) of porcine insulin in the experiment of cardiac arrested rat was almost the same as the calculated $K_m$.\(^1\)

RESULTS

Plasma Insulin and Serum Glucose Levels after Subcutaneous Administration

The changes in plasma immunoreactive insulin levels and serum glucose levels after subcutaneous administration of 0.2 U/kg of porcine and human insulin (10 or 25 μl) are illustrated in Fig. 2. For both injection volumes, plasma insulin levels increased rapidly and decreased gradually. The plasma insulin levels of human insulin were greater than those of porcine insulin after subcutaneous administration. The area under the curve (AUC) for human insulin was approximately 1.5 times of that for porcine insulin after subcutaneous administration. A significant difference was observed at 5 min after subcutaneous administration of 0.2 U/kg, 10 μl, and at 20 min after subcutaneous administration of 0.2 U/kg, 25 μl. The hypoglycemic effects of human insulin were also greater than those of porcine insulin after subcutaneous administration.

Plasma Insulin and Serum Glucose Levels after Intravenous Administration

The plasma insulin and serum glucose levels after intravenous administration of 0.2 U/kg

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FIG. 2. Changes in Plasma Insulin and Serum Glucose Levels after Subcutaneous Administration of Porcine and Human Insulin

A) Δ plasma IRI levels (0.2 U/kg, 10 μl), B) Δ plasma IRI levels (0.2 U/kg, 25 μl), C) % change in serum glucose levels (0.2 U/kg, 10 μl), D) % change in serum glucose levels (0.2 U/kg, 25 μl). Each point represents the mean value of 4–8 experiments. Vertical bars indicate SEM. a) and b) indicate statistical significance (a, $p<0.05$ and b, $p<0.001$).

● porcine insulin, ○ human insulin.
porcine and human insulin are illustrated in Fig. 3. The plasma insulin levels decreased rapidly after intravenous administration. No difference was observed in plasma insulin and serum glucose levels between the two kinds of insulins. AUC and hypoglycemic effects of both insulins after intravenous administration were greater than those of both insulins after subcutaneous administration.

Degradation of Insulin at Injection Site of Cardiac Arrested Rats

Table I shows the percent change in insulin levels of porcine and human insulin at the subcutaneous injection site of cardiac arrested rats at 10 min post-injection. No absorption was assumed to occur since the systemic circulation was completely stopped. Thus, the decreases in insulin levels in this system indicate degradation at the injection site. The human insulin levels were demonstrated to be greater than porcine insulin levels in the subcutaneous injection site of cardiac arrested rats.

Degradation and Absorption of Insulin in the Subcutaneous Injection Site of Anesthetized Rats

Figure 4 shows the gel filtration pattern of radioactivity extracted from subcutaneous injection site of anesthetized rats obtained 10 and 20 min post-injection. Most of the radioactivity was eluted at the position of insulin, with small peaks at the void volume and at the position of monoiodotyrosine. The insulin peaks of human insulin were greater than those of porcine insulin, and LMWP peaks of porcine insulin were greater than that of human insulin.

To clarify this phenomenon, time courses for the clearance of porcine and human insulin and

![Graph showing changes in plasma insulin and serum glucose levels.](image)

**FIG. 3. Changes in Plasma Insulin and Serum Glucose Levels after Intravenous Administration of 0.2 U/kg Porcine and Human Insulin**

Upper panel is Δ plasma IRI levels and lower panel is % change in serum glucose levels. Each point represents the mean value of 3-5 experiments. Vertical bars indicate SEM. Symbols as in Fig. 1.

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**TABLE I. Degradation of 0.2 U/kg Porcine and Human Insulin in Subcutaneous Injection Site of Cardiac Arrested Rats**

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Injection volume (µl)</th>
<th>Undegraded insulin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine</td>
<td>10</td>
<td>78.8 ± 2.4</td>
</tr>
<tr>
<td>Human</td>
<td>10</td>
<td>87.5 ± 0.9</td>
</tr>
<tr>
<td>Porcine</td>
<td>25</td>
<td>75.6 ± 2.6</td>
</tr>
<tr>
<td>Human</td>
<td>25</td>
<td>86.8 ± 1.0</td>
</tr>
</tbody>
</table>

The values represent the mean ± SEM of 4-7 animals in each group. a) and b) indicate statistical significance (a, p < 0.05 and b, p < 0.001).
formation of its metabolites at the subcutaneous injection site of anesthetized rats were investigated. Figure 5 shows a logarithmic plot of insulin, LMWP, and HMWP levels remaining at the injection site. Since a straight line was obtained for the insulin, a first-order process seems to be predominant for the clearance of insulin from the subcutaneous injection site. The levels of LMWP and HMWP of porcine insulin were greater than those of human insulin in the subcutaneous injection site.

The Kinetic Parameters for Porcine and Human Insulin in Subcutaneous Injection Site of Anesthetized Rats

Using these data, absorption and degradation rate constants of these substances were estimated according to a one-compartment model, and kinetic parameters are listed in Table II. The degradation rate constants of porcine and human insulin to LMWP were 0.0131 and 0.0077 min⁻¹, which were approximately half of the degradation rate constant of porcine insulin. Similar results were obtained when both insulins were administrated subcutaneously at a injection volume of 25 μl. The absorption rate constant of human insulin in subcutaneous injection site was greater than that of porcine insulin. These constants of both insulins of the 25 μl administration were greater than those of the 10 μl administration.

DISCUSSION

Present study was undertaken to compare the degradation and absorption of porcine and

![Gel Filtration Pattern of Radioactivity Extracted from Subcutaneous Tissue Samples 10 min and 20 min after Porcine and Human Insulin Injection of 0.2 U/kg, 10 μl Subcutaneously](image)

A) porcine insulin (10 min), B) human insulin (10 min), C) porcine insulin (20 min), D) human insulin (20 min). Gel: Toyopearl HW-55 F (1× 48 cm), elute: 3 M guanidine-hydrochloride in 2.4 M formic acid (1 ml/min).
FIG. 5. Disappearance of 0.2 U/kg, 10 μl and 25 μl Porcine and Human Insulin in Subcutaneous Injection Site
A) porcine insulin (0.2 U/kg, 10 μl), B) human insulin (0.2 U/kg, 10 μl), C) porcine insulin (0.2 U/kg, 25 μl), D) human insulin (0.2 U/kg, 25 μl). Each point represents the mean value of 4—5 experiments.
Vertical bars indicate SEM. Each line represents the curve fitted with the one-compartment model.
• insulin, ○ LMWP, △ HMWP.
human insulin at the subcutaneous injection site. Schou (mice)\textsuperscript{13} and Berger \textit{et al.} (pig)\textsuperscript{14} reported the disappearance of the subcutaneously injected various drugs and insulin using the subcutaneous clearance method. We demonstrated that the plasma insulin levels and hypoglycemic effect of human insulin were greater than those of porcine insulin after subcutaneous administration (0.2 U/kg). No difference was observed in the plasma insulin and serum glucose levels between the two kinds of insulins after intravenous administration. Keen \textit{et al.}\textsuperscript{15} reported that the human insulin had a slightly greater hypoglycemic effect at low dose and a slightly smaller effect at high dose when compared with porcine insulin. Ebihara \textit{et al.}\textsuperscript{20} noted that the $AUC$ for serum human insulin after 0.05 U/kg subcutaneous administration was greater than that for serum porcine insulin, but no difference was observed in the $AUC$ after 0.1 U/kg subcutaneous administration and intravenous administration. We also observed the small difference in plasma insulin and serum glucose levels between the two kinds of insulins in high dose (0.5 U/kg), (data not shown). These findings indicated that the degradation would be a dose dependent process. We found that the $AUC$ and the hypoglycemic effect of porcine and human insulins after intravenous administration were greater than those of both insulins after subcutaneous administration. The bioavailability of insulin injected subcutaneously was lower than expected, suggesting that insulin is degraded at the subcutaneous injection site.\textsuperscript{1,14,16}

In the kinetical analysis for porcine and human insulin after subcutaneous administration, we observed that the absorption rate constant of human insulin greater than that of porcine insulin, and that the degradation rate constant of human insulin is approximately half of that for porcine insulin. Galloway \textit{et al.}\textsuperscript{4} reported that the absorption of human insulin was faster than that of porcine insulin after subcutaneous administration. Sonnenberg \textit{et al.}\textsuperscript{17} suggested that subcutaneous absorption of human insulin was faster than that of porcine insulin and/or that of human insulin was degraded to a lesser degree than porcine insulin in normal subjects, which supported our results.

The absorption rate constants of porcine and human insulin administered at a injection volume of 25 $\mu$l were greater than those of at 10 $\mu$l. We suspect that this phenomenon occurred by the increase of hydrostatic pressure in the subcutaneous injection site. Using data of the plasma insulin levels, the absorption rate and

<table>
<thead>
<tr>
<th>Insulin</th>
<th>$V$ $\mu$l</th>
<th>Kinetic parameters (min$^{-1}$) $\pm$ uncertain sigma</th>
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</thead>
<tbody>
<tr>
<td>Porcine</td>
<td>10</td>
<td>$K_a$: 0.0208 $\pm$ 0.0029, $K_b$: 0.107, $K_c$: 0.0113, $K_m$: 0.0131 $\pm$ 0.0024, $K_h$: 0.0022 $\pm$ 0.0012</td>
</tr>
<tr>
<td>Human</td>
<td>25</td>
<td>$K_a$: 0.0253 $\pm$ 0.0034, $K_b$: 0.113, $K_c$: 0.0113, $K_m$: 0.0104 $\pm$ 0.0028, $K_h$: 0.0029 $\pm$ 0.0012</td>
</tr>
</tbody>
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\textit{Kinetic parameters were calculated by nonlinear least-squares regression based on Marquardt method. The weighting function used was 1. $K_b$ and $K_c$ were fixed. a) Injection volume ($\mu$l).}
the fraction of the administered dose that was absorbed following subcutaneous administration were estimated according to a one-compartment model with first-order absorption process. Similar results were obtained when both insulins were estimated by the subcutaneous clearance method.

The present study suggests that human insulin is more stable than porcine insulin against proteolytic enzymes in subcutaneous tissue of the rat. The efforts have been made to characterize the pattern of the proteolytic enzymes and the pathways of proteolysis in human and various animal skins.14) The various proteolytic enzymes were obtained in the skin of human, rat, cat, dog, pig, and other animals, and those enzymes show the same character with those in humans and other animals. Thus, it was suggested that human insulin should be more stable even in human subcutaneous tissue. Further research of the degradation of porcine and human insulin in humans is now in progress.

Acknowledgement We wish to thank Professor A. Ebihara, Department of Clinical Pharmacology, Oita National Medical School, for his kind advice.

This study was supported by grant no. 58870105 from the Ministry of Education, Science and Culture of Japan.

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