Clinical Pharmacology of Human Insulin of Recombinant DNA Origin in Healthy Volunteers

AKIO EBIHARA, KAZUOKI KONDO, KUROICHI OHASHI, KINORI KOSAKA, TAKESHI KUZUYA, AND SEIICHIRO TARUI

The serum insulin, fractional absorption, serum human C-peptide, and plasma glucose responses of normal fasting subjects were compared after the subcutaneous and intravenous administration of human insulin (recombinant DNA) and Novo Actrapid insulin. While no statistical difference at each time point was observed between the two insulins, for each time point after 0.05 and 0.1 U/kg s.c., the 0–6 h area under curve (AUC) after the 0.05 U/kg dose was greater for human insulin than for pork insulin. There was no difference in the 0–6 h AUC after the 0.1 U/kg s.c. dose. The serum human C-peptide responses to the two insulins were virtually identical. With the 0.05 U/kg s.c. dose, hypoglycemic effect of human insulin was greater than for Actrapid. This difference did not occur after 0.1 U/kg s.c. Following intravenous administration using 0.05 U/kg, the serum IRI, serum C-peptide, and glucose responses were the same. These data indicate only slight differences between human insulin and Actrapid insulin. DIABETES CARE 5 (SUPPL. 2): 35–38, 1982.

Since human insulin was produced by recombinant DNA technology, many efforts have been made to prove its potential clinical advantages over beef and porcine insulin. In this study we report the comparative effects of the human and porcine insulin on the plasma IRI, glucose, and C-peptide.

SUBJECTS AND METHODS

The study was divided into two parts, a subcutaneous (s.c.) study and an intravenous (i.v.) study. The experimental subjects for the s.c. study were six healthy male volunteers, aged 23–41 yr, weighing 60–72 kg; there were six healthy male volunteers, aged 20–40 yr, weighing 54–70 kg for the i.v. study.

All subjects had never received insulin before and had no family history of diabetes mellitus. A 75-g oral glucose tolerance test, EKG, chest x-ray, and blood chemistry were within the normal range in all subjects.

Informed written consent was obtained from each volunteer before inclusion in the study.

The test drug was the neutral regular formulation of human insulin (recombinant DNA) and the control drug was Actrapid Monocomponent Insulin (Novo). The total nitrogen and hypoglycemic potencies (rabbit assay) of the human and porcine insulins were comparable. Both insulins were administered by bolus injection at two dose levels, 0.05 and 0.1 U/kg for the s.c. study, and 0.025 and 0.05 U/kg for the i.v. study. The site of injection was 10 cm from the umbilicus toward the anterior superior iliac spine for the s.c. study and the antecubital vein for the i.v. study. Signs and symptoms were carefully observed and blood was sampled in succession to determine IRI, glucose, and C-peptide for 6 h after s.c. and for 4 h after i.v. injection.

Serum IRI and C-peptide were determined by radioimmunoassay[1–3] and plasma glucose by the glucose-oxidase method.[4]

All data were analyzed by the paired *t* test between human and porcine insulins; the area under the time-concentration curve from 0 to 6 h after injection (AUC 0–6) of plasma IRI was calculated by the trapezoidal method.

The standard curve for radioimmunoassay for IRI was identical for both human and porcine insulins.

RESULTS

The changes in serum IRI, plasma glucose, and serum C-peptide after the s.c. administration of 0.05 U/kg of the test insulins are demonstrated in Figure 1 and after 0.1 U/kg s.c. in Figure 2. For both dose levels serum IRI increased rapidly and decreased gradually. While no statistically significant differences between the two insulins were observed when the

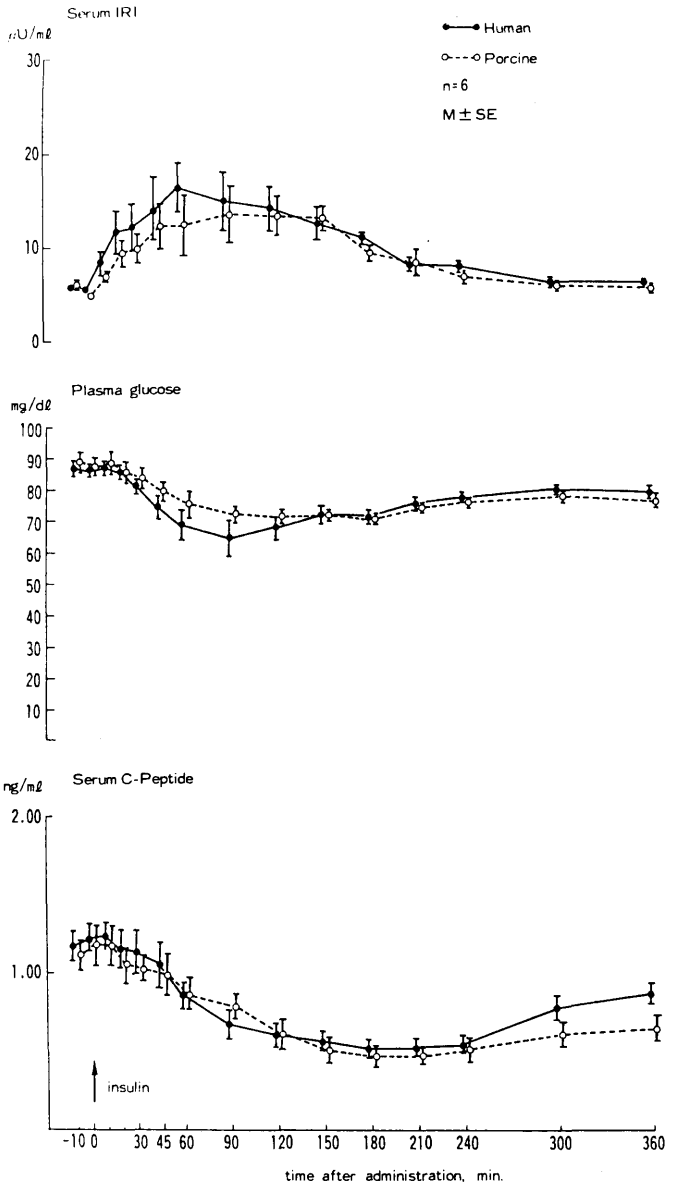

FIG. 1. Effect of insulin (0.05 U/kg, s.c.) on serum IRI, plasma glucose, and serum C-peptide.

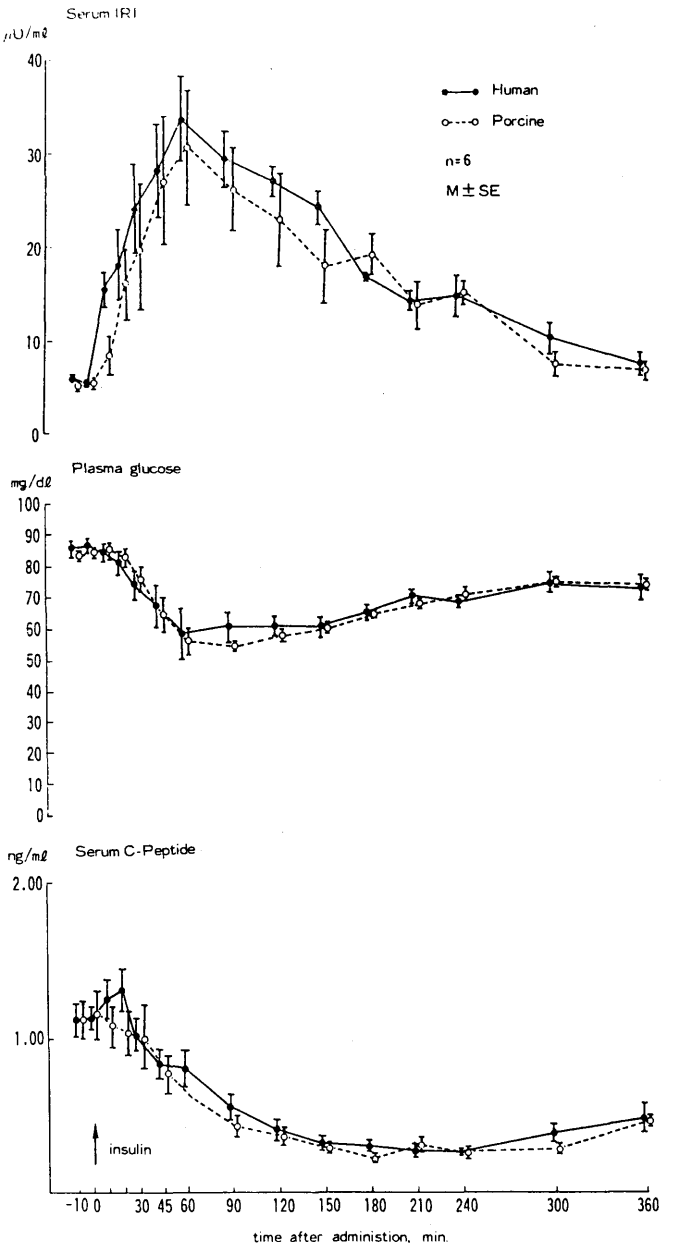

FIG. 2. Effect of insulin (0.1 U/kg, s.c.) on serum IRI, plasma glucose, and serum C-peptide.

responses for each time point were compared, for the 0.05 U/kg dose the area under the 0–6 h curve (AUC) for human insulin was greater for human than for pork insulin. This difference was not evident for the 0.1 U/kg dose.

After the injection of the test insulins, serum C-peptide concentrations decreased and remained depressed for 6 h. The suppression of C-peptide was the same after the two insulins.

Plasma glucose fell to its nadir 1.5 h after insulin had been injected. The hypoglycemic response to 0.05 U/kg s.c. of human insulin was greater for human than for porcine insulin. This difference did not occur with 0.1 U/kg s.c.

Tingling at injection site was complained of by four volunteers after s.c. injection of 0.1 U/kg porcine insulin, but not after s.c. injection of the same dose of human insulin.

Several subjects complained of hypoglycemic symptoms such as palpitation and cold sweating after both types of insulin.

The data after i.v. injection of 0.025 U/kg insulin are

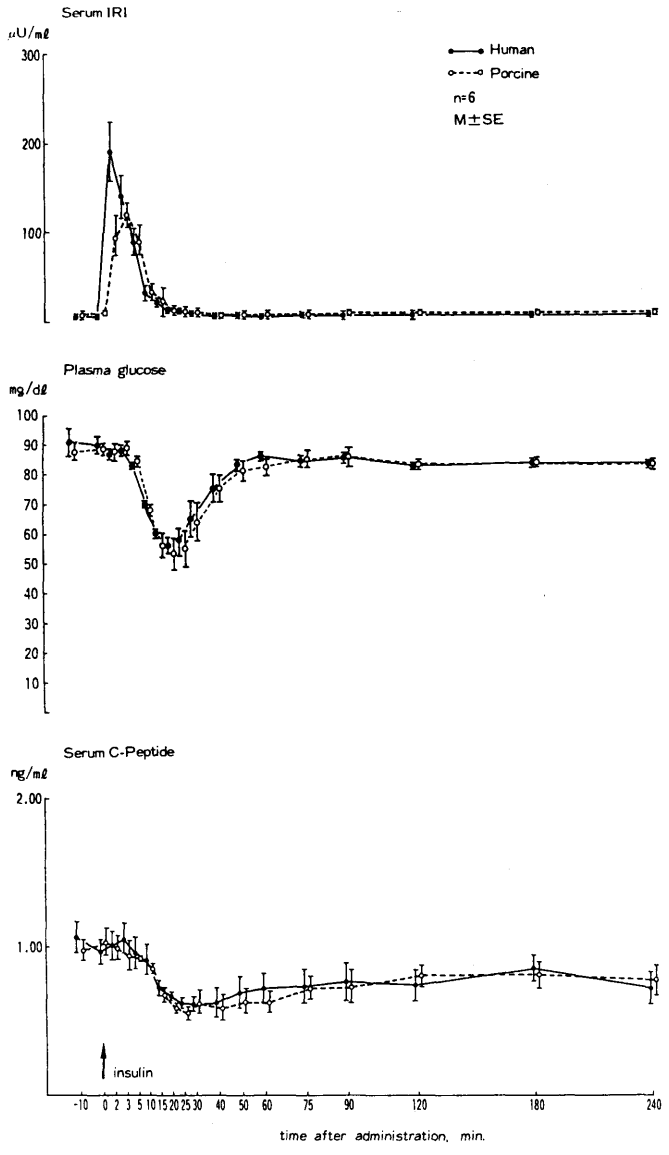

FIG. 3. Effect of insulin (0.025 U/kg, i.v.) on serum IRI, plasma glucose, and serum C-peptide.

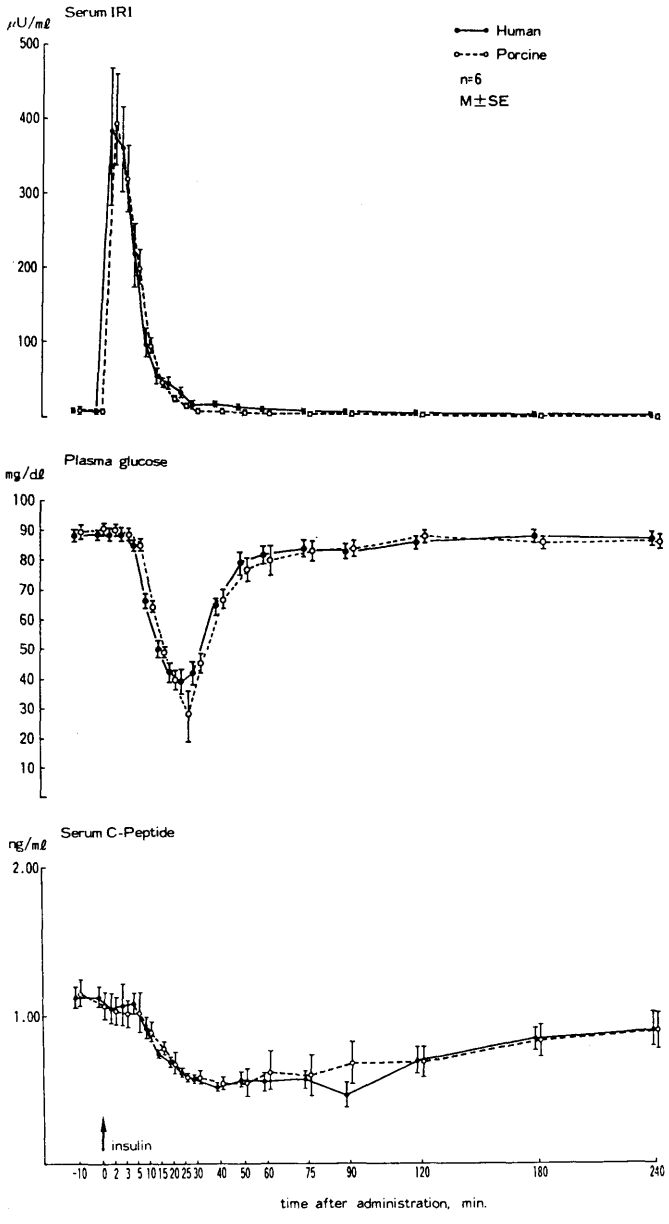

FIG. 4. Effect of insulin (0.05 U/kg, i.v.) on serum IRI, plasma glucose, and serum C-peptide.

demonstrated in Figure 3 and those after s.c. injection of 0.05 U/kg insulin in Figure 4.

Both the serum IRI and plasma glucose decreased rapidly, with the nadir of plasma glucose occurring about 25 min after injection. No significant differences between the two insulins were observed in serum IRI, AUC 0–6 of IRI, plasma glucose, or serum C-peptide after 0.025 U/kg or 0.05 U/kg.

Apart from hypoglycemic symptoms such as cold sweating, palpitation, and a burning sensation after both insulins, no other unwanted reactions were observed. Abnormal findings were not documented in health examinations performed 1 wk after each study.

DISCUSSION

This study was performed to elucidate the pharmacokinetic and pharmacologic differences, if any, between human insulin produced by recombinant DNA technology and conventional porcine insulin.

Human insulin administered by bolus s.c. and i.v. injections showed essentially the same pharmacokinetics and pharmacologic effects as porcine insulin in healthy volunteers.

Our failure to demonstrate striking differences between human and porcine insulin are consistent with other reports that have disclosed either minimal or no differences between the two insulins. Thus, Massi-Benedetti et al.[5] and Galloway et al.[6] reported that human insulin of recombinant DNA origin showed the same hypoglycemic activity with porcine insulin in normal human beings.

Keen et al.[7] reported that the human insulin had a slightly greater blood glucose-reducing effect at low doses and slightly smaller effects at high doses when compared with porcine insulin. Like Keen et al., we observed a greater level of IRI and a greater blood glucose-reducing effect after s.c. injection of human insulin than after s.c. injection of porcine insulin. Such differences were not observed after the i.v. application of both forms of insulin.

In agreement with Galloway et al.[8] we have demonstrated that serum concentrations of human insulin may be higher than after porcine insulin. Unlike Galloway et al.[8] we found that fractional absorption of human insulin was greater than that of porcine insulin after s.c. administration. Specifically, we observed that the 0–6 h AUC for serum IRI for 0.05 U/kg s.c. was greater for human than for porcine insulin. Our estimates of fractional absorption assumed that the contribution to measured serum IRI by endogenous insulin was the same after human and porcine insulin since the pattern of human C-peptide suppression was the same after the two insulins. On the other hand, in their estimates of fractional absorption, Galloway et al.[8] attempted to correct for the contribution of endogenous insulin to their serum IRI measurements.

Using our methods, we conclude that the bioavailability of subcutaneously injected human insulin seemed to be greater than that of subcutaneously injected porcine insulin. The greater bioavailability can explain the greater blood glucose-reducing effect of human insulin when compared with porcine insulin.

Federlin et al.[9] reported that the human insulin reached higher serum concentrations and induced lower blood glucose levels after s.c. injection than porcine insulin.

We have obtained similar results with human insulin produced by enzymatic conversion from porcine insulin in comparison with conventional porcine insulin.[10] It seems to be natural because both insulins are structurally identical.

Although the difference observed in this study was small, it may be of clinical importance when it is applied for a long-term use.

From the Department of Clinical Pharmacology, Oita National Medical School (A.E. and K.O.); the Departments of Clinical Pharmacology (K.K.) and Endocrinology (T.K.), Jichi Medical School; the Department of Internal Medicine, Osaka University School of Medicine (S.K.).

Address reprint requests to A. Ebihara, Oita National Medical School, Idaigaoka, Hazama-cho, Oita-gun, Oita-ken, 879-56 Japan.

REFERENCES

[1] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin-two antibody system. Diabetes *12*: 115–26, 1963.

[2] Hales, C. N., and Randle, P. J.: Immunoassay of insulin with insulin antibody precipitate. Biochem. J. *88*: 137–46, 1963.

[3] Nakagawa, N., et al.: A basic study of the development of a C-peptide radioimmunoassay kit. Radioisotopes *25*: 271–76, 1976.

[4] Sasaki, K.: Ultramicroquantitative determination of blood glucose with orthotoluidin-nitric acid method. Comparison with orthoaminobiphenyl method and orthotoluidine method. Rinsho Byori *12*: 434–37, 1964.

[5] Massi-Benedetti, M., Burrin, J. M., Capaldo, B., and Alberti, K. G. M. M.: A comparative study of the activity of biosynthetic human insulin and pork insulin using the glucose clamp technique in normal subjects. Diabetes Care *4*: 163–67, 1981.

[6] Galloway, J. A., Spradlin, C. T., Root, M. A., and Fineberg, S. E.: The plasma glucose response of normal fasting subjects to neutral regular and NPH biosynthetic human and purified pork insulins. Diabetes Care *4*: 183–88, 1981.

[7] Keen, H., Glynne, A., Pickup, J. C., Viberti, G. C., Bilous, R. W., Jarrett, R. J., and Marsden, R.: Human insulin produced by recombinant DNA technology: safety and hypoglycemic potency in healthy men. Lancet *2*: 398–401, 1980.

[8] Galloway, J. A., Root, M. A., Bergstrom, C. T., Howey, D. C., Fineberg, S. E., and Jackson, R. L.: Clinical pharmacologic studies with human insulin (recombinant DNA). Diabetes Care 5 (Suppl. 2): 13–22, 1982.

[9] Federlin, K., Laube, H., and Velcovsky, H. G.: Biologic and immunologic in vivo and in vitro studies with biosynthetic human insulin. Diabetes Care *4*: 170–74, 1981.

[10] Ebihara, A., Kondo, K., Ohashi, K., Kosaka, K., Kuzuya, T., and Matsuda, A.: Comparative clinical pharmacology of human and porcine insulin in normal subjects. Am. J. Med. In press.