Pharmaceutical Research, Vol. 6, No. 9, 1989

Report

Enhanced Bioavailability of Subcutaneously Injected Insulin Coadministered with Collagen in Rats and Humans

Ryohei Hori,[1] Fusao Komada,[2] Seigo Iwakawa,[1] Yutaka Seino,[3] and Katsuhiko Okumura[2,4]

Received January 9, 1989; accepted April 14, 1989

The present study was undertaken to develop an agent that stabilizes insulin injected subcutaneously. ^{125}I-Porcine insulin with 0.2 U/kg unlabeled porcine insulin was subcutaneously injected with or without collagen in the rat under the depilated skin of the back. At various times, the radioactivity in subcutaneous tissue was assayed for insulin and its metabolites by gel filtration. The degradation and absorption rate constants of insulin at the subcutaneous injection site were estimated according to a one-compartment model. The degradation rate constant of insulin in the presence of collagen at the injection site was less than half of the control rate. The inhibition was confirmed by increases in the immunoreactive insulin plasma levels and the hypoglycemic effect in rats and healthy volunteers. We postulate that collagen prevents insulin from being degraded by inhibiting proteolytic enzymes, mainly collagenase-like peptidase, in subcutaneous tissue.

KEY WORDS: insulin; collagen; injection; subcutaneous injection; bioavailability.

INTRODUCTION

The degradation of subcutaneously injected insulin, which has been found to occur in the rat (1–3) and pig (4), might account for the higher dose requirements when insulin is administered subcutaneously rather than intravenously in man (5,6). We have reported that benzyloxycarbonyl-Gly-Pro-Leu-Gly (ZP; substrate of collagenase-like peptidase) amplifies the bioavailability and hypoglycemic effect of insulin by inhibiting local degradation of exogenous insulin at the subcutaneous injection site (3). Further, the degradation of insulin was suppressed by an inhibitor (phosphoramidon) or substrate (ZP) of collagenase-like peptidase in subcutaneous tissue *in vitro* (7). Collagen is extensively investigated as biomaterial and has been clinically studied in drug delivery systems (8) and in conjunction with a hemodialyzer (9). Atelo collagen has little antigenic activity, because it is free of the telopeptide region, which is found to be the strongest and most important antigenic determinant (10). In the present study, atelo collagen (a substrate of collagenase-like peptidase) was used to stabilize injected insulin in the subcutaneous tissue.

MATERIALS AND METHODS

Materials. Monocomponent porcine insulin (26.0 U/mg; Novo Industri A/S, Denmark) and atelo collagen (Koken, Japan) were used. ^{125}I-insulin was prepared by modification of the chloramine-T method (11) to yield monoiodinated insulin.

Procedure. Male Wistar rats weighing 125–150 g were anesthetized with pentobarbital and the rectal temperature was kept constant (36.5 ± 0.5°C) by setting the animals in a temperature-constant room (3,12). Using a microsyringe with a thin needle (N-733, Hamilton, U.S.A.), 10 μl of isotonic phosphate buffer solution (pH 7.0) containing ^{125}I-insulin (0.01 μCi/rat) and unlabeled insulin (0.2 U/kg) with or without atelo collagen was injected subcutaneously in a single dosage to each rat under the depilated skin of the back. At various times, skin samples and subcutaneous tissue around the injection site were taken for analysis. The analytical method by gel filtration (1.5 × 48 cm; Toyopearl HW-55, Toyo Soda, Japan) was described in our previous reports (3,12). To measure the plasma immunoreactive insulin levels (13) or serum glucose levels (14), unlabeled porcine insulin was injected subcutaneously or intravenously to fasting (16 hr) rats. Diabetes was induced in the rat by intravenous injection of streptozotocin 50 mg/kg (15). The diabetic rat displayed polyuria, glycosuria, and significant hyperglycemia (mean serum glucose, 450 mg/dl) at the time of the experiment (3 weeks after injection of streptozotocin).

Cardiac Arrest Model. To estimate the degradation of insulin at the injection site when its absorption would be negligible, the degradation during cardiac arrest was investigated. This condition was induced by direct injection of pentobarbital (200 mg/kg) to the heart 5 sec before the experiment (3,12). No absorption was assumed to occur since the systemic circulation was stopped completely. The degradation rate constant was 0.0137 min^{-1}, which was almost the same as the calculated value using the *in vivo* experiment. The degradation rate of insulin in the cardiac arrested

[1] Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan.
[2] Department of Hospital Pharmacy, School of Medicine, Kobe University, Chuou-ku, Kobe 650, Japan.
[3] Department of Clinical Nutrition, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan.
[4] To whom correspondence should be addressed.

0724-8741/89/0900-0813$06.00/0

rat confirmed that the calculated degradation rate *in vivo* was a reasonable value (3,12).

Kinetic Model in Vivo. The disposition of insulin at the subcutaneous injection site in the anesthetized rat was analyzed by assuming a one compartment model as described previously (3,12). Insulin is biotransformed to low molecular weight products (LMWP) and high molecular weight products (HMWP; mixtures of subcutaneous components bound to undegraded insulin and insulin degradation products) (16), with a degradation rate constant (k_m) and HMWP formation rate constant (k_h), respectively. Insulin, LMWP, and HMWP are independently absorbed into systemic circulation with absorption rate constants (k_a, k_b, and k_c, respectively). The amount of insulin in the injection site (X), amount of LMWP in the injection site (L), and amount of HMWP in the injection site (H) were expressed by (17)

$$X = X_0 e^{-kt} \tag{1}$$

$$L = \frac{X_0 k_m}{k - kb}(e^{-k_b t} - e^{-kt}) + L_0 e^{-k_b t} \tag{2}$$

and

$$H = \frac{X_0 k_h}{k - k_c}(e^{-k_c t} - e^{-kt}) + H_0 e^{-k_c t} \tag{3}$$

where X_0 is the initial amount of insulin, L_0 is the initial amount of LMWP, H_0 is the initial amount of HMWP, $k = k_a + k_m + k_h$, and t is the sampling time. Data of X, L, and H were fitted to Eqs. (1)–(3) by nonlinear least-squares regression based on Marquardt method (18). k_b and k_c were previously determined by subcutaneously injecting LMWP and HMWP, respectively (3,12).

Study in Healthy Volunteers. Three healthy volunteers (within 10% of ideal body weight; 25–42 years of age) fasted overnight. Insulin (0.1 U/kg) with or without atelo collagen (0.5 μg/kg) was administered subcutaneously in the morning. Plasma levels of glucose, plasma immunoreactive insulin, and C peptide were monitored for 4 hr after administration. Plasma C-peptide concentration was measured by radioimmunoassay kit (Daiichi Radioisotope Laboratories, Japan).

RESULTS AND DISCUSSION

Table I shows the changed percentage of insulin administered with or without atelo collagen at the injection site in cardiac arrested rats for 10 min after injection. No absorption was assumed to occur since the systemic circulation was stopped completely. Thus, the decrease in insulin levels in this system indicates degradation at the injection site. By the coadministration of atelo collagen, insulin degradation was significantly decreased, and a dose-dependent inhibiting tendency by coadministered atelo collagen was indicated in this method.

Table I. Degradation of Insulin for 10 min with or Without Atelo Collagen at the Injection Site in Cardiac Arrested Rats[a]

	N	Undegraded insulin (%)
Insulin (0.2 U/kg)	8	78.0 ± 2.2
With atelo collagen (1 μg/kg)	6	84.5 ± 1.5*
With atelo collagen (100 μg/kg)	5	86.6 ± 1.8**

[a] Degradation was analyzed by gel filtration. Values are expressed as mean ± SE.
* $P < 0.05$.
** $P < 0.02$.

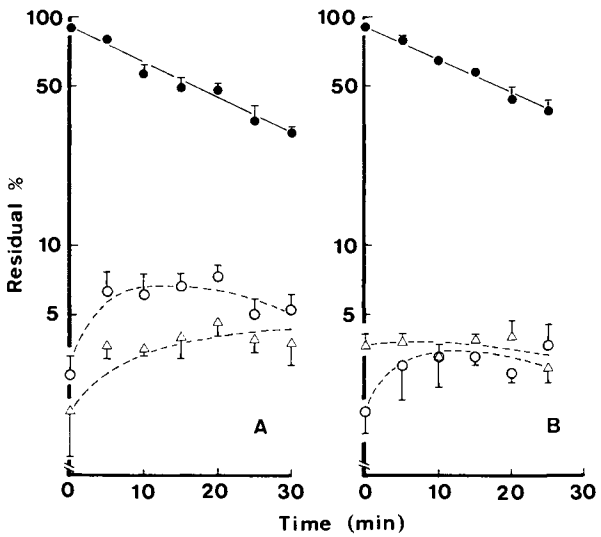

Fig. 1. Disappearance of insulin with or without atelo collagen at the subcutaneous injection site. (A) Insulin (0.2 U/kg); (B) with atelo collagen (100 μg/kg). Each point represents the mean value of five experiments. Vertical bars indicate SE. Each line represents the curve fitted with the one-compartment model. ●, Insulin; ○, LMWP; △, HMWP.

The time courses for the local clearance of insulin with or without atelo collagen and the formation of its metabolites at the injection site in anesthetized rats are shown in Fig. 1. Since a straight line was obtained for the insulin clearance, an apparent first-order process is assumed for the local clearance of insulin from the injection site. Little insulin degradation occurred when insulin was coadministered with atelo collagen. Using these data, absorption and degradation rate constants of insulin were estimated according to a one-

Table II. Effect of Atelo Collagen (100 μg/kg) on Kinetic Parameters of Insulin (0.2 U/kg) in the Subcutaneous Injection Site[a]

	Kinetic parameter (min^{-1})				
	k_a	k_b	k_c	k_m	k_h
Insulin	0.0208	0.107	0.0113	0.0131	0.0022
With collagen	0.0273	0.107	0.0113	0.0060	0.0005

[a] k_a, k_b, and k_c are absorption rate constants of insulin, LMWP, and HMWP from the injection site, respectively. k_m and k_h are the degradation rate constant of insulin and production rate constant of HMWP in the injection site, respectively. k_b and k_c were determined by subcutaneously injecting LMWP and HMWP, respectively, in the previous experiments (3,12). The mean data at each time point were fitted by nonlinear least-squares regression based on the Marquardt method. Weighting function used was 1. k_b and k_c were fixed. The equations for this one-compartment model are described under Materials and Methods.

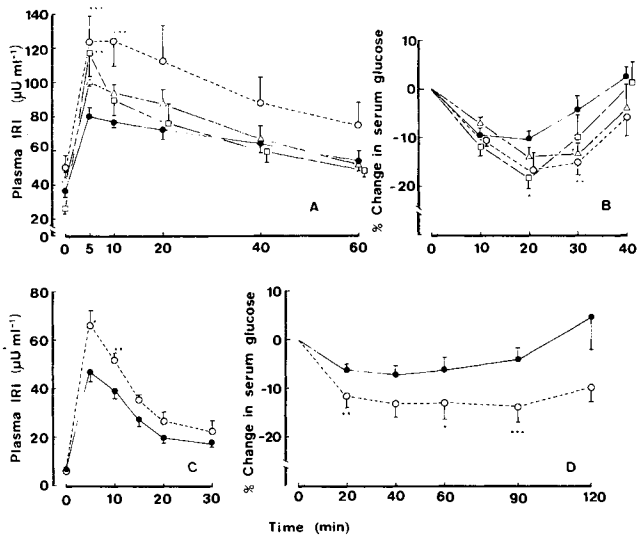

Fig. 2. Effect of atelo collagen on plasma IRI levels and hypoglycemic effect of subcutaneously injected insulin in normal and diabetic rats. (A) Plasma IRI levels in normal rats; (B) percentage change in serum glucose levels in normal rats; (C) plasma IRI levels in diabetic rats; (D) percentage change in serum glucose levels in diabetic rats. Each point represents the mean value of 6–12 experiments. Vertical bars indicate SE. ●, Insulin (0.2 U/kg); ○, with atelo collagen (100 μg/kg); △, with atelo collagen (10 μg/kg); □, atelo collagen (1 μg/kg). Statistical significance: (*) $P < 0.05$; (**) $P < 0.02$; (***) $P < 0.01$.

compartment model (3), and the estimated parameters are shown in Table II. The degradation rate constant (k_m) of insulin in the presence of atelo collagen was less than half of the control.

In our previous report (7), insulin degrading activity which is inhibited by Z-Gly-Pro-Leu-Gly (ZP), a collagenase-like peptidase substrate, is located in the 160,000*g* supernatant in subcutaneous tissue *in vitro*. We assume that atelo collagen as a collagenase-like peptidase substrate also inhibits competitively the degradation of insulin by collagenase-like peptidase in injection site.

Recently, Chipkin *et al.* (19) reported the potentiation of the hypoglycemic effect of insulin by coadministration of thiorphan, an enkephalinase inhibitor. Malfroy and Schwartz (20) found that enkephalinase activity was inhibited by phosphoramidon. We showed that phosphoramidon prevented the degradation of insulin in subcutaneous tissue *in vitro* (7). Thiorphan might inhibit other enzymes that have a substrate specificity similar to that of enkephalinase, because the thiorphan dose (30 and 100 mg/kg) was much higher than that of atelo collagen (1, 10, and 100 μg/kg) in this report.

The absorption rate constant (k_a) of insulin in the presence of atelo collagen was slightly greater than that of the control. In our previous report (3), we showed that Z-Gly-Pro-Leu-Gly (ZP) significantly increased the absorption rate constant for insulin at the subcutaneous injection site. Williams *et al.* (21) reported that aprotinin, a trypsin and kallikrein inhibitor, causes local sustained hyperaemia at the subcutaneous injection site, as measured by plethysmography. Berger *et al.* (22) reported that aprotinin enhances the bioavailability of subcutaneously injected insulin by increasing the blood flow near the injection site. Hence, it is postulated that atelo collagen and ZP influence local circulation.

The plasma immunoreactive insulin (IRI) levels and hypoglycemic effect of insulin in normal and diabetic rats are illustrated in Fig. 2. When insulin was injected with atelo collagen in normal and diabetic rats, the plasma IRI levels were significantly higher than those in the controls (Figs. 2A and C). Larger atelo collagen doses resulted in higher plasma IRI levels. The dose-dependent effects of coadministered atelo collagen were also observed in cardiac arrested rats. However, intravenous injection of insulin with atelo collagen did not cause significant changes in plasma IRI levels compared with those of the control (Table III). This result suggests that atelo collagen has no effect on the kinetics of insulin other than the kinetics in the absorption site.

The hypoglycemic effect of insulin in the presence of atelo collagen was also greater than that in the control in normal and diabetic rats (Figs. 2B and D).

From the area under the curve (AUC) of plasma IRI levels, the loss of insulin at the subcutaneous site prior to entering systemic circulation was estimated to be 40% of the dose in the absence of atelo collagen in diabetic rats. Insulin loss from the injection site was decreased to 16% of the dose by the presence of atelo collagen.

When insulin was coadministered with atelo collagen in healthy volunteers, the plasma IRI levels were higher than those of the control, and the hypoglycemic effect of insulin was also enhanced (Fig. 3). Insulin administration rapidly reduced the plasma C-peptide concentration. The C-peptide levels were decreased to 59 ± 3% (without atelo collagen) or 65 ± 5% (with atelo collagen) of the preadministered levels (1.5 ± 0.2 ng/ml) at 30 min and to less than 20% (with or without atelo collagen) at 120 min. The effect of atelo collagen on insulin secretion was insignificant, and the increases

Table III. Kinetic Parameters of Insulin (0.2 U/kg) After Intravenous Administration with or Without Atelo Collagen (100 μg/kg) in Diabetic Rats[a]

		Kinetic parameter			
	N	A (μU/ml)	B (μU/ml)	α (min^{-1})	β (min^{-1})
Insulin	6	2242 ± 199	332 ± 70	0.99 ± 0.21	0.34 ± 0.04
With collagen	4	2297 ± 221	324 ± 52	1.33 ± 0.14	0.27 ± 0.04

[a] Kinetic parameters were estimated according to two-compartment model using nonlinear least-squares regression based on the Marquardt method. Values are expressed as mean ± SE.

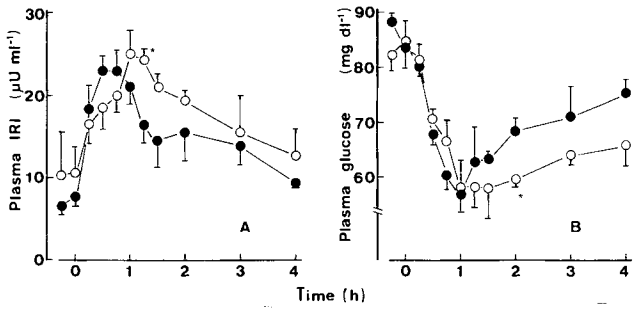

Fig. 3. Effect of coadministration of atelo collagen on serum IRI levels and hypoglycemic effect of subcutaneously injected insulin in healthy volunteers. Insulin (0.1 U/kg) with or without atelo collagen (0.5 μg/kg) was administered subcutaneously in three volunteers. (A) Serum IRI levels; (B) serum glucose levels. ●, Insulin; ○, insulin with atelo collagen. Statistical significance: (*) $P < 0.05$.

in plasma IRI and hypoglycemic effect were considered to be attributable to the absorbed insulin.

The study of the degradation of insulin at the subcutaneous injection site has provided a good understanding of the phenomenon involved, leading to exploration of this stabilizing agent (8,9). Several reports have shown that gelatin and its derivatives cause histamine release and allergic and/or anaphylactic reactions (23,24). But atelo collagen has little antigenic activity, because it is devoid of the antigenic region. An increase by atelo collagen of the biological effect of subcutaneously injected insulin in patients could be therapeutically important.

REFERENCES

1. M. Berger, P. A. Halbon, W. A. Muller, R. E. Offord, A. E. Renold, and M. Vranic. *Diabetologia* 15:133–140 (1978).
2. R. W. Stevenson, T. I. Tsakok, and J. A. Parsons. *Diabetologia* 18:423–426 (1980).
3. R. Hori, F. Komada, and K. Okumura. *J. Pharm. Sci.* 72:435–439 (1983).
4. M. Berger, P. A. Halbon, L. Girardier, J. Seydoux, R. E. Offord, and A. E. Renold. *Diabetologia* 17:97–99 (1979).
5. S. M. O. Guerra and A. E. Kitabchi. *J. Clin. Endocrinol. Metab.* 42:869–874 (1976).
6. T. Kobayashi, S. Sawano, T. Itho, K. Kosaka, H. Hirayama, and Y. Kasuya. *Diabetes* 32:331–336 (1983).
7. F. Komada, K. Okumura, and R. Hori. *J. Pharmacobio-Dyn.* 8:33–40 (1985).
8. A. L. Rubin, K. H. Stenzel, T. Miyata, M. J. White, and M. Dunn. *J. Clin. Pharmacol.* 13:309–312 (1973).
9. K. H. Stenzel, J. F. Sullivan, T. Miyata, and A. L. Rubin. *Trans. Am. Soc. Artif. Intern. Organs* 15:114–117 (1969).
10. K. H. Stenzel, T. Miyata, and A. L. Rubin. *Annu. Rev. Biophys. Bioeng.* 3:231–253 (1974).
11. P. Freychet, J. Roth, and D. M. Neville Jr. *Biochem. Biophys. Res. Commun.* 43:400–408 (1971).
12. K. Okumura, F. Komada, and R. Hori. *J. Pharmacobio-Dyn.* 8:25–32 (1985).
13. C. N. Hales and P. J. Randle. *Biochem. J.* 88:137–146 (1963).
14. A. Hyvärinen and E. A. Nikkilä. *Clin. Chim. Acta* 7:140–143 (1962).
15. A. Junod, A. E. Lambert, W. Stauffacher, and A. E. Renold. *J. Clin. Invest.* 48:2129–2139 (1969).
16. R. Hori, Y. Saito, M. Yasuhara, and K. Okumura. *J. Pharmacobio-Dyn.* 7:910–916 (1984).
17. M. Gibaldi and D. Perrier. In J. Swarbrick (ed.), *Drug and the Pharmaceutical Sciences, Vol. I, Pharmacokinetics,* Marcel Dekker, New York, 1975, pp. 1–27.
18. T. Nakagawa, Y. Koyanagi, and H. Togawa. *SALS,* Library Program of the University of Tokyo Computer Center, Tokyo (1975).
19. R. E. Chipkin, W. Kreutner, and W. Billard. *Eur. J. Pharmacol.* 102:151–154 (1984).
20. B. Malfloy and J. C. Schwartz. *Biochem. Biophys. Res. Commun.* 106:276–285 (1982).
21. G. Williams, J. C. Pickup, S. Bowcock, E. Cook, and H. Keen. *Diabetologia* 24:91–94 (1983).
22. M. Berger, H. J. Cüppers, P. A. Halban, and R. E. Offerd. *Diabetes* 29:81–83 (1983).
23. W. Lorenz, A. Doenicke, K. Messmer, H. J. Reimann, M. Thermann, W. Lahn, J. Berr, A. Schmal, P. Dormann, P. Regenfuss, and H. Hamelmann. *Br. J. Anaesth.* 48:151–164 (1976).
24. A. Doenicke, B. Grote, and W. Lorenz. *Br. J. Anaesth.* 49:681–688 (1977).