Plasma Insulin and C-Peptide After Subcutaneous and Intravenous Administration of Human Insulin (recombinant DNA) and Purified Porcine Insulin in Healthy Men

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We have compared the plasma profiles of C-peptide, human insulin (recombinant DNA), and purified porcine insulin of pancreatic origin (PPI) in six nondiabetic men after low-dose (4.8 U) or high-dose (9.6 U) subcutaneous injection and low-rate (1.0 U/h) or high-rate (1.7 U/h) intravenous infusion. There was no significant difference in plasma C-peptide or glucose levels when human insulin was compared with PPI at either dose level for subcutaneous injection or intravenous infusion. Thus, there was equal suppression of endogenous insulin by the two species of exogenous insulin. For low-dose subcutaneous injection there was no significant difference between plasma insulin levels of the two species at single time points or when areas were compared. For high-dose subcutaneous injection mean plasma insulin levels were higher after human insulin than after PPI (20–300 min); this serial difference reached conventional statistical significance at 50 min ($P < 0.05$) and 90 min ($P < 0.01$). For the area under plasma insulin profiles between 0 and 90 min after high-dose s.c. injection, human insulin was higher than PPI ($P = 0.06$). There was no significant difference between insulin concentrations after human insulin and PPI given by either low- or high-dose intravenous infusion, except that high-dose PPI values (55–110 min) were slightly but significantly higher after high-dose intravenous infusion. Further comparisons of the pharmacokinetics of human and other species of insulin are, therefore, justified in larger numbers of subjects and particularly in diabetic individuals. DIABETES CARE 5 (SUPPL. 2): 29–34, 1982.

There appears to be little difference in hypoglycemic[1–3] or antilipolytic[4] potency in normal man of intravenously or subcutaneously administered purified porcine insulin (PPI) of pancreatic origin and human insulin produced by either recombinant DNA technology or semisynthesis from the porcine molecule. However, preliminary work[5,6] in normal man suggested that human insulin was more rapidly absorbed from the subcutaneous site than was PPI. This was not confirmed by a recent study[3] of semisynthetic human insulin injected subcutaneously at one dose level into healthy men.

In this article we examine the plasma concentrations of insulin and C-peptide after low- or high-dose subcutaneous injection or intravenous infusion of human insulin or PPI into nondiabetic man.

MATERIALS AND METHODS

The experimental subjects and design, materials used, and methods of administration were as described in a previous publication[1] that reported plasma glucose responses to human and porcine insulin. In brief, six normal men received, in random order, an abdominal subcutaneous injection of insulin on each of four occasions. The injection volume was 0.24 ml, containing either 4.8 or 9.6 U of purified porcine (PPI) or human insulin of the same protein concentration (by protein nitrogen determination). Subjects were fasted overnight and blood samples taken (for immediate glucose and subsequent plasma insulin and C-peptide measurement) through an indwelling venous cannula. Sampling times are shown in Figures 1 and 2, with preinjection samples at −20 and −10 min.

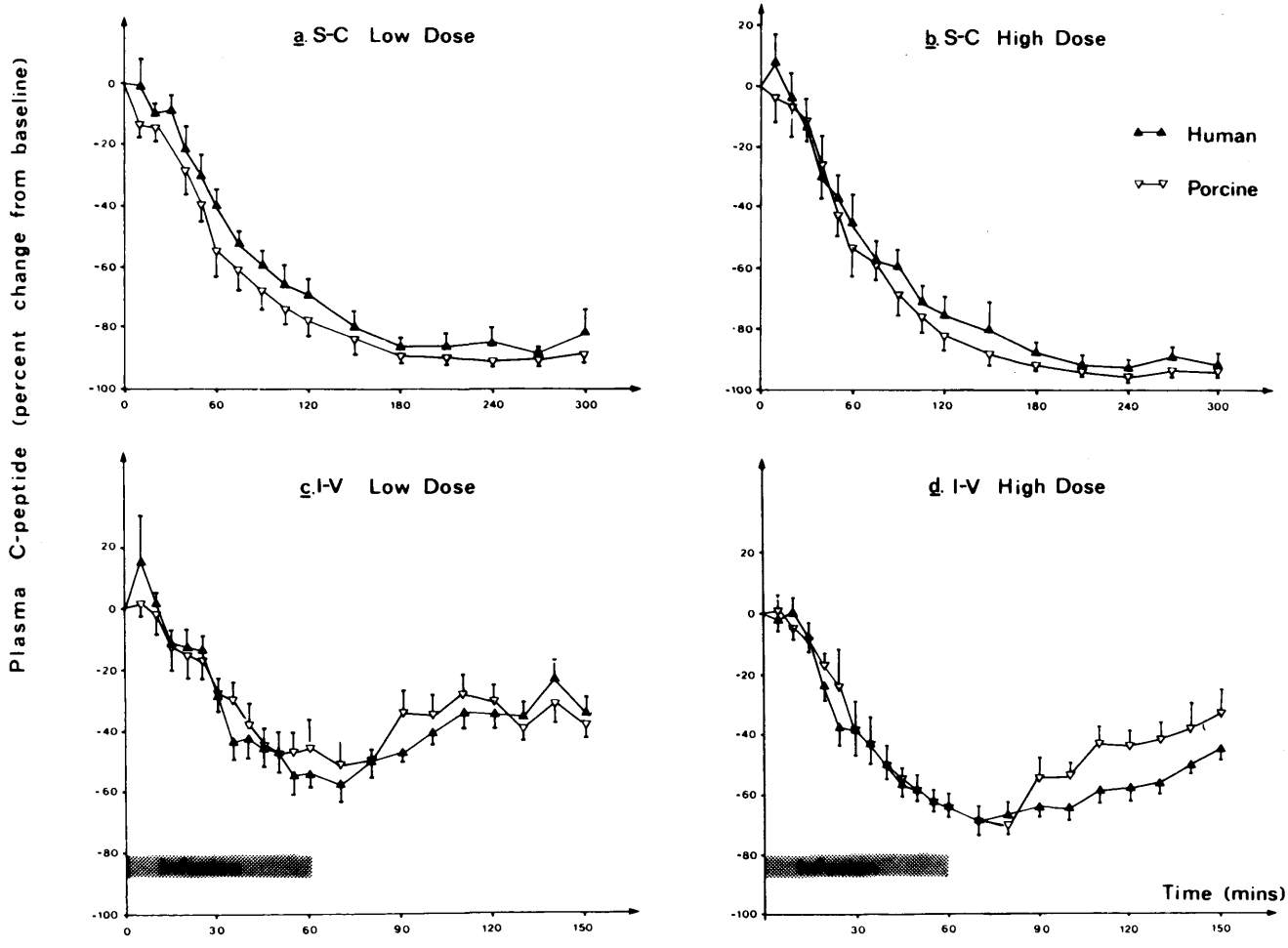

FIG. 1. Mean ± SEM plasma C-peptide concentrations (percent change from baseline) in six normal men after subcutaneous low-dose (panel a) or high-dose (panel b) porcine or human insulin injection, and low-dose (panel c) or high-dose (panel d) porcine or human insulin intravenous infusion. Injection was given at time 0, intravenous infusion was from time 0 to 60 min, as indicated.

Six other normal men received an intravenous infusion of insulin, on each of four occasions, into an intravenous cannula inserted in the nonsampling arm. Insulin solutions were infused for 1 h from an IVAC 630 piston pump at a fixed rate of 50 ml/h, delivering either 1.0 or 1.7 U/h of PPI or human insulin, again standardized by protein concentration. Adsorption of insulin to the plastic reservoir bag was prevented by adding 7.0 ml of plasma from the subject to each 500-ml bag of sterile 0.154-mol/L saline before the addition of the insulin (checked by experiments with ^{125}I-insulin).[1] Blood samples for glucose, insulin, and C-peptide were taken at the time points indicated in Figures 1 and 2, with preinfusion samples at −10 and −5 min. Heparinized blood samples were immediately centrifuged at 4°C and the plasma frozen and stored at −40°C for later assay.

Radioimmunoassay of plasma insulin[7] concentrations were read against curves obtained using human insulin standard concentrations for all baseline preinjection or preinfusion samples and for samples after human insulin administration experiments; porcine insulin standards were used for assay of samples in post-PPI administration experiments only. PPI and human insulin standard curves were identical in the low range. The exact concentrations in the stock insulin standard solutions were calculated from the optical density at 276 nm. Standards ranged from 0.05 to 10 ng/ml. Tracer was ^{125}I-porcine insulin, labeled by the lactoperoxidase method and purified by high-performance liquid chromatography to yield a monoiodinated, tyrosine-A_{14}–labeled product. Insulin concentrations are presented as ng/ml (1 ng ~ 0.17 nmol ~ 25 μU). Plasma C-peptide was measured by radioimmunoassay.[8] Individual values are the means of duplicate estimations. Plasma glucose concentrations were measured by a glucose-oxidase method (Auto-Analyzer AA2, Technicon, Tarrytown, New York).

Statistical significance of differences was assessed by analysis of variance.

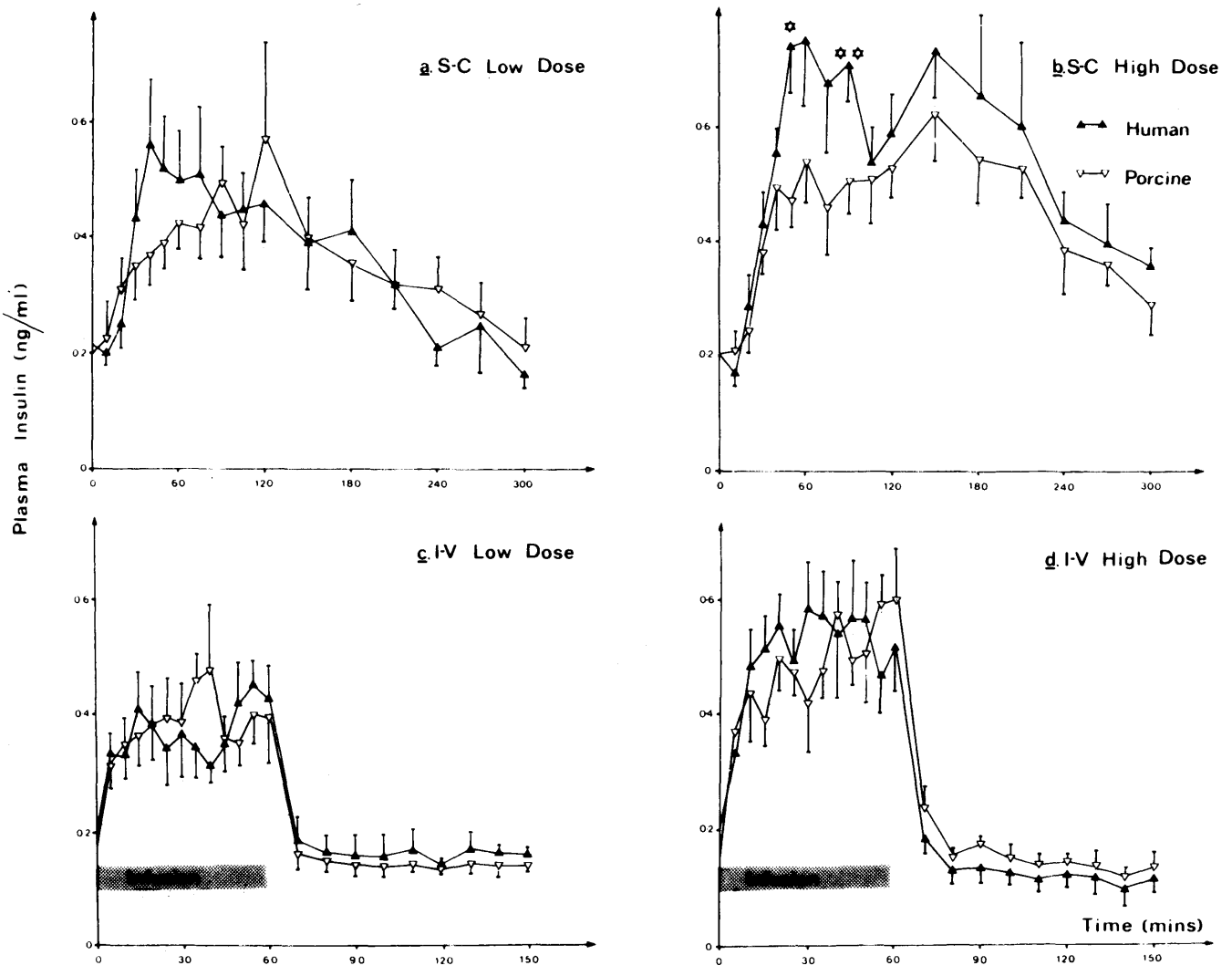

*FIG. 2. Mean ± SEM plasma insulin concentrations in six normal men after subcutaneous low-dose (panel a) or high-dose (panel b) porcine or human insulin injections and low-dose (panel c) or high-dose (panel d) porcine or human insulin intravenous infusion. Injection was given at time 0, intravenous infusion was from time 0 to 60 min, as indicated. * = point significantly different from porcine insulin mean value at the same time ($P < 0.05$); **, $P < 0.01$.*

RESULTS

Plasma C-Peptide Concentrations

Figure 1 shows the mean plasma C-peptide concentrations from the subjects given high- and low-dose subcutaneous injections or high- and low-dose intravenous infusions of PPI or human insulin. Results are expressed as the percentage change from the baseline since the fall is highly correlated with the baseline value. The actual baseline values were (mean ± SD): s.c. high-dose human insulin: 0.60 ± 0.30, PPI: 0.84 ± 0.25; s.c. low-dose human insulin: 0.79 ± 0.25, PPI: 0.79 ± 0.30; i.v. high-dose human insulin: 0.57 ± 0.28, PPI: 0.54 ± 0.15; low-dose human insulin: 0.50 ± 0.11, PPI: 0.51 ± 0.13 pmol/ml. There was no significant difference in plasma C-peptide concentrations at any time point when experiments with porcine insulin were compared with human insulin experiments, at either dose level, for subcutaneous or intravenous administration of the insulin.

Plasma Glucose Concentrations

Tables 1 and 2 show the mean ± SD plasma glucose concentrations after high- and low-dose subcutaneous injections and high- and low-dose intravenous infusion of PPI or human insulin. There was no significant difference between plasma glucose values at any single time point, when the porcine insulin experiment was compared with the human insulin study, at either dose level, or route of administration. There was, thus, no evidence for differential suppression of endog-

TABLE 1
Plasma glucose concentrations (mmol/L) after low-dose (4.8 U) or high-dose (9.6 U) human or porcine subcutaneous insulin injection

Time (min)	Human insulin				Porcine insulin			
	Low dose		High dose		Low dose		High dose	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline*	5.80	0.34	5.46	0.52	5.73	0.29	5.76	0.29
10	5.68	0.23	5.47	0.53	5.63	0.29	5.62	0.25
20	5.59	0.26	5.32	0.57	5.53	0.30	5.50	0.26
30	5.38	0.22	5.18	0.55	5.54	0.25	5.38	0.21
40	5.10	0.36	4.86	0.61	5.27	0.22	5.09	0.25
50	4.76	0.51	4.50	0.71	4.97	0.33	4.78	0.43
60	4.37	0.72	4.01	0.93	4.70	0.41	4.37	0.90
75	4.11	0.79	3.76	0.88	4.34	0.59	4.03	1.04
90	3.98	0.43	3.75	0.68	4.07	0.68	4.05	0.61
105	3.81	0.31	3.72	0.63	3.96	0.73	3.72	0.63
120	3.80	0.36	3.62	0.62	3.96	0.75	3.52	0.51
150	3.64	0.34	3.64	0.62	3.92	0.72	3.41	0.43
180	3.84	0.44	3.77	0.66	3.83	0.66	3.73	0.49
210	4.00	0.49	3.78	0.56	4.27	0.53	3.69	0.47
240	4.25	0.65	3.77	0.71	4.27	0.50	3.85	0.59
270	4.44	0.61	3.96	0.59	4.47	0.49	3.99	0.67
300	4.68	0.44	4.06	0.45	4.55	0.43	4.14	0.63

*Baseline plasma glucose = mean of values at −20, −10, and 0 time points.

TABLE 2
Plasma glucose concentrations (mmol/L) after a 1-h low-dose (1.0 U/h) or high-dose (1.7 U/h) intravenous infusion of human or porcine insulin

Time (min)	Human insulin				Porcine insulin			
	Low dose		High dose		Low dose		High dose	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	5.0	0.28	5.57	0.35	5.64	0.35	5.58	0.31
0	5.45	0.26	5.53	0.39	5.61	0.32	5.56	0.29
5	5.53	0.26	5.51	0.24	5.59	0.30	5.52	0.31
10	5.44	0.27	5.46	0.24	5.51	0.31	5.39	0.32
15	5.31	0.27	5.27	0.31	5.45	0.31	5.27	0.30
20	5.12	0.26	5.10	0.40	5.27	0.25	5.00	0.32
25	5.00	0.36	4.83	0.47	5.15	0.27	4.76	0.35
30	4.92	0.33	4.57	0.52	4.99	0.28	4.43	0.42
35	4.74	0.36	4.36	0.56	4.90	0.30	4.19	0.47
40	4.52	0.38	4.12	0.59	4.74	0.32	4.01	0.47
45	4.44	0.43	3.88	0.67	4.73	0.29	3.78	0.51
50	4.41	0.48	3.78	0.65	4.64	0.32	3.68	0.43
55	4.30	0.38	3.60	0.68	4.55	0.35	3.48	0.53
60	4.26	0.41	3.53	0.66	4.59	0.36	3.38	0.52
70	4.34	0.43	3.72	0.63	4.58	0.43	3.45	0.47
80	4.60	0.43	4.25	0.57	4.89	0.35	4.07	0.42
90	4.78	0.26	4.65	0.54	5.04	0.43	4.75	0.38
100	4.88	0.32	4.90	0.43	5.13	0.33	4.90	0.25
110	5.07	0.24	5.02	0.39	5.13	0.35	5.01	0.18
120	5.03	0.21	5.15	0.31	5.15	0.41	5.04	0.16
130	5.12	0.17	5.17	0.28	5.17	0.30	5.09	0.16
140	5.12	0.14	5.20	0.32	5.23	0.29	5.16	0.13
150	5.16	0.18	5.16	0.27	5.20	0.26	5.19	0.18

enous insulin by the two species, either directly or through effects on plasma glucose decrement.

Plasma Insulin Concentrations

Figure 2 compares the mean plasma insulin concentrations from the subjects given human insulin and PPI after high- and low-dose subcutaneous injections or during high- and low-dose intravenous infusions.

Low-dose subcutaneous injection. For low-dose subcutaneous injection there was no significant difference between the plasma insulin concentration estimates with the two species of insulin, either at single time points or in comparisons of areas under the concentration curves.

High-dose subcutaneous injection. For high-dose subcutaneous injection mean plasma insulin levels were higher after human insulin than after PPI administration, from 20–300 min. This serial difference reached statistical significance at 50 min ($P < 0.05$) and 90 min ($P < 0.01$). The mean areas under the concentration curves for the whole experimental period were not significantly different for the two species of insulin. However, for the area between time 0 and 90 min, significance reached a value of $P = 0.06$, PPI versus human insulin. Using both low- and high-dose values the area (0–90 min) was higher for human than porcine insulin, with $P = 0.05$.

Low- or high-dose intravenous infusion. For low- or high-dose intravenous infusion plasma insulin concentrations did not differ significantly between the two insulin species except that the high-dose porcine values were slightly but significantly higher between 55 min and 110 min ($P < 0.05$ at 55, 60, 70, and 100 min and $P < 0.01$ at 90 and 110 min). After stopping the infusion, the rate of fall of plasma insulin was the same for both insulins.

Dose Differences

After subcutaneous injection plasma concentrations of insulin were significantly higher after high than after low doses (for both species of insulin) by the areas under the concentration curves up to 300 min ($P < 0.02$) or when increments above baseline at individual time points were compared (90 min, $P < 0.05$; 150 min, $P < 0.01$; 210 min, $P < 0.05$; 240 min, $P < 0.05$; 270 min, $P < 0.05$; 300 min, $P < 0.001$).

For intravenous data, in both species peak plasma insulin values were significantly higher after high- than after low-dose administration and also when increments above baseline were compared at individual time points ($P < 0.05$ at 10, 20, 25, 40, 50, 55, and 60 min; $P < 0.01$ at 35, 45, and 70 min).

DISCUSSION

In this study plasma insulin profiles were similar after porcine or human insulin, whether administered by subcutaneous injection or by intravenous infusion. There was, however, a trend to higher human plasma insulin values after high-dose subcutaneous injection, becoming statistically significant at 50 and 90 min and showing in a greater area under the human insulin profile between 0 and 90 min. Equal degrees of C-peptide suppression by the two species show that these differences are unlikely to be due to differential suppression of endogenous insulin secretion. These results are of interest in view of the two previously published reports[5,6] showing more rapid absorption of subcutaneously injected human insulin (recombinant DNA). In contrast, semisynthetic human insulin was recently[3] shown to produce identical plasma profiles to porcine insulin when injected subcutaneously at a dose level of 0.075 U/kg into healthy men. This difference may be due to the use of somatostatin to suppress endogenous insulin in the semisynthetic human insulin study, with, perhaps, concomitant alteration in subcutaneous blood flow and modulation of absorption. Alternatively, there may be dose-related absorption, lower doses of subcutaneous insulin masking a possible preferential absorption of the human insulin preparation. In this respect, the present study pointed more clearly to differences after injection of 9.6 U (approximately 0.15 U/kg) and the study of Botterman et al.[5] used 0.3 U/kg.

It is also of interest that C-peptide levels reached their nadirs well after plasma insulin values reached their peaks, reflecting, perhaps, the longer half-life of C-peptide in the circulation. Further, subcutaneous insulin suppressed C-peptide release much more strongly than intravenous infusion, despite the generation of similar plasma insulin concentrations.

There is large inter- and intrasubject variation in the rate of insulin absorption after subcutaneous injection.[9] This will blur any real difference in absorption rates between insulin species, and large numbers of observations will be required before differences can be excluded with confidence. Even if the small differences described here are real, they are so small as to seem unlikely to be of clinical significance. In the present study a decrease in plasma glucose was the same after human insulin and PPI. However, it will be of great interest to study the pharmacokinetics in insulin-dependent diabetic individuals, in whom circulating anti-insulin antibodies may modify absorption.[10] This may assume importance if the long-term therapeutic use of human insulin in human diabetic individuals proves this preparation to be less antigenic than even highly purified porcine insulin. Locally induced variation in subcutaneous blood flow[11,12] and possibly enzymatic destruction of insulin after subcutaneous injection[13] may also be important factors governing absorption of insulin in diabetic individuals and thus indications for further comparisons of human and porcine insulin pharmacokinetics.

The similarity of the plasma insulin profiles during intravenous infusion of human or porcine insulin are not unexpected. This similarity gives weight to the possibility of real absorption differences after subcutaneous injection, which was followed by differing profiles of insulinemia. The comparable rates of fall of plasma human and porcine insulin after cessation of intravenous infusion indicate that the intravenous half-life for the two molecular species is similar. The small, but significantly higher levels of PPI after high-dose infusion are unexplained.

In conclusion, these studies justify the continued com-

parison of the pharmacokinetics of human and other species of insulin in larger numbers of subjects and particularly in insulin-dependent diabetic subjects.

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