CLINICAL RESEARCH

Absorption of isophane (NPH) insulin and its clinical implications

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Abstract

Absorption of ^{125}I -NPH insulin (^{125}I -isophane insulin) (40 IU/ml) was studied in eight diabetics given 50% and 150% of their normal daily dose of insulin. Insulin absorption correlated with plasma insulin ($r=0.97$, $p<0.001$) and blood glucose ($r=-0.87$, $p<0.01$) concentrations. Absorption was slower at higher doses, so that trebling the insulin dose only doubled the amount absorbed over the first 24 hours. The plasma elimination half time ($t_{1/2}$) of insulin was about five minutes. Thus, the disappearance of radiolabelled insulin is a reliable and quantitative index of insulin absorption; subcutaneous degradation, if present, is minimal and constant. Changes in dose of intermediate-acting insulin further increases the large variation in insulin absorption. This implies that minor adjustments of intermediate insulin dosage are probably futile.

Introduction

Most studies of insulin absorption have been performed with radiolabelled insulin preparations. The disappearance of radioactivity from the injection site is assumed to indicate that the insulin has been absorbed. This technique has shown enormous variability in absorption both within and between individuals.¹⁻⁴ Intra-individual variation in absorption of intermediate-acting insulins may account for 80% of the day-to-day variation in blood glucose concentration and thus be the single most important factor in the unstable control of diabetes.⁵ Although the absorption characteristics of insulin labelled with radio-

iodine are similar to those of unlabelled insulin,¹ the validity of this technique has been questioned. It has been argued that a substantial and variable proportion of insulin given subcutaneously is degraded in situ.^{7,8} If this is the case residual radioactivity at the injection site would represent fragmented as well as intact insulin molecules and thus be an unreliable index of the amount of biologically active insulin entering the circulation.

Our study had two aims: firstly, to validate the radiolabelled insulin technique by correlating the disappearance of radioactivity from the subcutaneous injection site with the appearance of immunoreactive insulin in plasma and blood glucose concentrations; and, secondly, to examine the effect of the size of the dose on the absorption characteristics of NPH insulin.

Patients and methods

Eight diabetic patients treated with insulin (four men and four women), aged 22-49 years, gave informed consent to the study. All were within 20% of ideal body weight. One had minimal endogenous insulin secretion with postprandial C-peptide concentrations of 0.2 nmol/l; the others had no endogenous insulin secretion. All had low insulin antibody titres (insulin-binding below 7%). Further details of the patients are given in table I. None had nephropathy or local lipodystrophy. No patients had illnesses other than diabetes.

The patients were admitted to hospital for three days, having omitted the evening dose of insulin the day before admission. On each study day the total daily dose of insulin was given as a single subcutaneous injection of NPH insulin (40 IU/ml) in the thigh at 0700. The usual daily dose (46 ± 12 IU, mean $\pm$ SD) was given on day 1 (table I), 55% of this dose (25 ± 7 IU) on day 2, and 145% (67 ± 17 IU) on day 3 (table II). The ^{125}I -NPH was made from highly purified crystalline porcine insulin (Nordisk Gentofte) and labelled before pharmaceutical preparation by the triiodine method.³ The preparation was monoiodinated, contained 80% unlabelled insulin, and had a specific radioactivity of 10 $\mu\text{Ci}/40$ IU. The biological and immunological properties of the preparation were indistinguishable from the NPH insulin (Insulatard).³ The patients were pretreated with potassium iodide to prevent accumulation of radioiodine in the thyroid. The pattern of diet and exercise was exactly the same during the three study days.

The disappearance of ^{125}I -NPH was measured by external counting

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of residual radioactivity over the injection site.¹ The precision of the measurement of radioactivity over the injection site is above 95% when more than 2% of the initial radioactivity is present and the accuracy is above 97%.¹ Measurements were made 0, 1½, 3, 6, 9, 15, and 24 hours after injection on day 1 and at 1½-hourly intervals throughout days 2 and 3 until less than 2% of radioactivity was present at the injection site or until the end of the experiment.

Immunoreactive insulin was determined in unextracted plasma⁹ as insulin-binding was low (table I). Immunoreactive insulin and blood glucose concentrations were measured every 1½ hours on days 2

The half time for insulin in plasma ($t_{1/2}$) was calculated for each patient for both day 2 and day 3 during the period in which a steady insulin absorption rate and plasma insulin concentration was obtained (1000-1430, fig 2). We used the formula $t_{1/2} = Vd \times Cp / 1.4 \times Ar^{10}$ (Cp being the mean insulin concentration during the steady-state period, Vd the distribution volume for insulin (0.146 ml/g¹¹), and Ar the calculated mean insulin absorption rate during the period).

Statistical analysis was by one-way analysis of variance, parametric regression analysis, and Wilcoxon's paired rank-sum test. Levels of significance were $p \leq 0.05$ (two-tailed).

TABLE I—Details of eight patients studied

Case No	Age (years)	Sex	Duration of insulin treatment (years)	Insulin-binding to antibodies (%)	Insulin dose (IU/24h)		Weight index ¹⁷	Complications
					Morning	Evening		
1	33	F	10	3.2	28 (NPH) 16 (Soluble)	16 (NPH) 4 (Soluble)	1.00	Background retinopathy Neuropathy
2	29	M	5	2.9	24 (NPH) 12 (Soluble)	12 (NPH) 4 (Soluble)	1.18	Nil
3	31	F	15	4.0	28 (NPH) 8 (Soluble)	12 (NPH)	1.05	Background retinopathy Neuropathy
4	48	M	15	3.0	22 (NPH) 6 (Soluble)	14 (NPH) 4 (Soluble)	1.12	Background retinopathy
5	22	F	18	3.0	26 (NPH) 10 (Soluble)	16 (NPH) 6 (Soluble)	1.04	Neuropathy
6	49	M	17	7.2	18 (NPH) 4 (Soluble)	14 (NPH)	1.02	Background retinopathy Neuropathy
7	42	M	28	2.1	28 (NPH)	10 (NPH)	1.03	Background retinopathy
8	23	F	19	3.0	20 (NPH)	10 (NPH)	0.97	Background retinopathy

TABLE II—Data on insulin absorption

Case No	Injected insulin (IU)			Absorbed insulin (IU/24h)			Mean blood glucose concentration (mmol/l)		
	Day 2	Day 3	Increase	Day 2	Day 3	Change	Day 2	Day 3	Change
1	19	57	38	23.9	23.2	-0.7	15.7	18.6	2.9
2	20	54	34	22.3	36.5	14.2	16.5	15.4	-1.1
3	28	68	40	25.7	58.4	32.7	12.5	7.3	-5.2
4	16	44	28	15.6	20.1	4.5	14.1	17.1	3.0
5	36	92	56	44.4	64.9	20.5	15.0	8.9	-6.0
6	28	76	48	29.4	72.2	42.8	14.7	6.6	-8.1
7	32	90	58	37.6	51.1	13.5	16.9	12.3	-4.6
8	19	57	38	17.0	45.5	28.5	19.5	10.7	-8.8
Mean	24.8	67.3	42.5	27.0	46.5	19.5	15.6	12.1	-3.5
Range	16-36	44-92	24-58	15.6-37.6	20.1-64.9	-0.7-42.8	12.5-19.5	6.6-18.6	-8.8-2.9

and 3 but not during day 1, as residual effects from unlabelled NPH insulin injected on the days before admission could be expected.

For insulin-binding measurement 10 µl serum was incubated for 16-18 hours at 4°C with 200 µl 0.04 mol/l phosphate buffer (pH 7.4) containing 0.15 mol/l NaCl, 0.01 mol/l Na₂-EDTA, 5 g/l bovine albumin, 2.5 g/l bovine IgG, and 2.5 × 10⁴ counts/minute ¹²⁵I-A₁₄-monoiodo-insulin (specific radioactivity 250-300 µCi/µg). Separation of bound from free ligand was achieved by adding 1.4 ml of polyethylene glycol 6000 (15% w/v in 0.05 mol/l veronal buffer, pH 8.4, containing 0.1% v/v Tween 20). Binding is expressed as the percentage of ¹²⁵I bound and is considered specific if ≥ 4%, which represents the mean (+ 4 SD) of 10 normal sera. The standard deviation of independent duplicates was 0.14% binding for sera exerting specific binding.

CALCULATIONS

The absorption data are presented as (a) disappearance curves illustrating the remaining amount of radioactivity over the injection site at various times after injection of insulin (fig 1); (b) T_{50} —that is, the time by which the mean radioactivity over the injection site had declined to half the initial value; and (c) as insulin absorption rates—that is, as the amount of insulin absorbed in 1½ hours (fig 2) or during 24 hours (table II). The absorption of NPH insulin usually takes more than 24 hours and therefore insulin absorption rates during a given period were calculated by adding absorption rates from all injection sites which were still active. The absorption rate during the time period t_1 to t_2 from a single injection site was calculated as:

$$\frac{(cpm_{t_1} - cpm_{t_2})}{cpm_{t_0}} \times \text{dose IU}/t_2 - t_1$$

where cpm_{t_1} and cpm_{t_2} are the counts per minute at time t_1 and t_2 and cpm_{t_0} is the counts per minute immediately after the injection of the insulin dose.

FIG 1—Mean disappearance curves (± 1 SEM) illustrating the remaining amount of radioactivity above the injection site in eight insulin-dependent diabetics given 55% (●), 100% (▲), and 145% (*) of their usual insulin dose as ¹²⁵I-NPH insulin. The remaining amount of radioactivity differed significantly 3-24 hours after the injection of insulin ($p < 0.05-0.01$).

Results

Absorption of ¹²⁵I-NPH insulin—The mean disappearance curves for 55%, 100%, and 145% of the normal daily dose are shown in fig 1. The remaining amount of radioactivity at the injection site differed significantly between the three doses from 3-24 hours after the injection of insulin (one-way analysis of variance, $p < 0.05-0.01$). This had the effect of increasing the mean T_{50} from 12.0 hours for the 55% dose to 13.8 hours for the 100% dose and to 18.9 hours for the 145% dose ($p < 0.05$). Increasing the amount of insulin injected from a mean of

24.8 IU to 67.3 IU ($275 \pm 7\%$, mean ± 1 SEM) increased the amount of insulin absorbed from 27 IU to only 46.5 IU daily ($177 \pm 22\%$, $p = 0.01$) (table II). In fact one patient (case 1) did not absorb more insulin on day 3 despite an increase in the dose from 19 to 57 IU.

Plasma immunoreactive insulin—Mean plasma insulin concentrations and mean insulin absorption rates (IU/90 min) during days 2 and 3 are shown in fig 2. There was a significant correlation between the mean

FIG 2—Mean blood glucose and mean plasma insulin concentrations and mean insulin absorption rate (± 1 SEM) in eight patients given 55% (broken line) and 145% (solid line) of their usual insulin dose as a single morning injection of NPH insulin.

FIG 3—Correlation between the mean insulin absorption rate (IU/90 min) for each of the 32 14-hour periods during day 2 (▲) and 3 (●) and the mean plasma insulin concentration at the end of any given period. Significant limits of 99% for the correlation coefficient were 0.91–0.99.

amount of insulin absorbed in 90 min and the mean plasma insulin concentration at the end of any given period with 99% significance levels for the correlation coefficient of 0.91–0.99 (fig 3).

Half time for insulin in plasma—The half time ($t_{1/2}$) for insulin in plasma during the plateau period (1000 to 1430, fig 2) was 5.5 ± 0.7 minutes (mean ± 1 SEM) for day 2 and 4.8 ± 0.7 minutes for day 3; this difference was not significant.

Blood glucose concentrations—There were no episodes of severe hypoglycaemia when the dose of insulin was increased from 55 to

145% between days 2 and 3. The change in mean blood glucose concentration from day 2 to day 3 correlated with the change in absorbed amount of insulin from day 2 to day 3 ($r = -0.87$, $p < 0.01$, table II) but not with the change in the amount of insulin injected. The mean fasting morning blood glucose concentrations from 0400 to 0700 (fig 2) correlated closely with the mean insulin absorption rate (IU/90 min) ($r = -0.99$, $p < 0.001$) and with mean plasma insulin concentrations ($r = 0.94$, $p < 0.01$).

Discussion

A potential limitation of studies based on the rate of disappearance of radioiodinated insulin is that some, if not all, patients treated with insulin may degrade a substantial proportion of injected insulin before it enters the circulation.^{7,8} To resolve this problem we compared the disappearance rate of labelled insulin with changes in circulating concentrations of insulin and glucose in insulin-dependent patients without endogenous insulin secretion (one patient had minimal C-peptide secretion but was similar in all respects to the other patients). Mean insulin absorption rates correlated extremely well with mean plasma insulin and mean blood glucose concentrations. Fasting, early morning (0400–0700) blood glucose concentrations are relatively unaffected by variables such as diet and exercise and were strongly correlated with the mean insulin absorption rate and with mean plasma insulin concentrations. The half time (T_{50}) of insulin in plasma was about five minutes, corresponding to values during intravenous infusion of insulin.¹¹ These observations confirm that the radioiodine method is a reliable and quantitative index of insulin absorption and that subcutaneous degradation, if present, is minimal and constant. This is further confirmed by a strong correlation ($r = 0.94$, $p < 0.01$) between radioactivity and immunoreactive insulin in extracts of biopsies from the subcutaneous injection site of ¹²⁵I-NPH insulin in pigs.¹²

Little attention has been given to the finding that the absorption of insulin may vary with the size of dose.¹ We found that the disappearance of NPH insulin (40 IU/ml) from the site of injection was slower at higher doses, so that on average trebling the dose only doubled the absolute amount of insulin absorbed over 24 hours. Kølendorf *et al*³ found a mean half time of seven hours for NPH insulin (40 IU/ml) using a dose of 8 units. We found a half time of 12 hours with a mean dose of 25 units increasing to 19 hours with a mean dose of 67 units. This explains why clinical experience often disagrees with the timing shown on the charts provided by manufacturers of insulin. Patients can absorb more insulin than the amount injected in a single day (table II), since absorption of NPH insulin can occur 24 hours after injection (fig 1).

The different rate of absorption of NPH insulin from the injection site with changes in dose could be due either to changes in volume or to changes in the absolute amount of injected insulin, as the insulin concentration was unchanged. Further studies are needed to solve this problem. A crossover design was not used, so as to avoid hypoglycaemia on day 3 with the 145% dose. The blood glucose concentration was higher during the first part of day 3 than on day 2. This could result in increased subcutaneous blood flow¹³ which accelerates the disappearance of insulin from the injection site.¹² We have, therefore, possibly underestimated the slower absorption rate with higher doses.

The most striking feature of insulin absorption is that the process is very erratic, with coefficients of variation for half time around 25% within, and 50% between, individuals.^{1,3} Although soluble and intermediate insulins have equally variable absorption rates,¹ the clinical implications differ. Soluble insulin is used to provide short-lasting peaks in plasma insulin concentrations to meet meal-time requirements. A variation of 25% around a peak reached in—for example, 75 minutes—may still adequately control postprandial hyperglycaemia. Repeated injections of soluble insulin can provide very acceptable control during the day but not during the night.¹⁴ In contrast, inter-

mediate insulins are used to supply basal insulin requirements. A variation of 25% around a half time of 14 hours means that the same dose of insulin may produce hypoglycaemia on one day and good control or hyperglycaemia the next. Thus, the overnight period is the Achilles's heel of conventional insulin treatment,¹⁵ and much of the minor adjustment of dosage is probably futile. The intermediate insulins are not precision tools.

Since the rate of absorption of NPH insulin depends on the dose the time scatter, and hence the unpredictability of clinical effect, will increase with the size of dose. This explains the success of treatment schedules based on multiple injections of soluble insulin during the day with small doses of NPH insulin last thing at night.¹⁶ Soluble and intermediate insulin treatment given twice daily is not necessarily the treatment of choice for young insulin-dependent patients, and alternative regimens must be found if conventional treatment is to meet the challenge of portable infusion devices.

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Cyclosporin A as prophylaxis against graft-versus-host disease in 36 patients

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Abstract

Oral cyclosporin A was used as prophylaxis against graft-versus-host disease in (a) 31 patients with acute leukaemia or aplastic anaemia given transplants of HLA-matched bone marrow and (b) five patients with inborn errors of metabolism given transplants of haplotype-identical (parental) bone marrow. Twenty-six patients survived longer than two months after the operation. Despite the cyclosporin A, 31 patients (86%) suffered an acute form of graft-versus-host disease and 22 (61%) a chronic form. Nevertheless, the disease was usually treatable with

immunosuppressive agents and caused the death of only one patient.

Cyclosporin A caused renal toxicity in all cases; occasionally this was associated with a "capillary leak" syndrome, fatal in two patients. In children hypertension, fits, and fluid retention were common side effects. Blood concentrations of cyclosporin A correlated with blood urea values and blood pressure but did not predict the occurrence of graft-versus-host disease. Four different dose schedules were used to find the optimum way to administer the drug.

Oral cyclosporin A is extremely effective at reducing the severity of graft-versus-host disease, but prevention of the disease is limited by toxicity of the drug and variable absorption. Better results might be achieved with parenteral administration or by using the drug in combination with other methods.

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Introduction

Graft-versus-host disease is a major complication of bone marrow transplantation. The disease is not completely understood but appears to be initiated by mature donor T lymphocytes.¹ The new immunosuppressive agent cyclosporin A, which