SHORT REPORTS

Change to U-100 insulin does not appear to affect insulin absorption

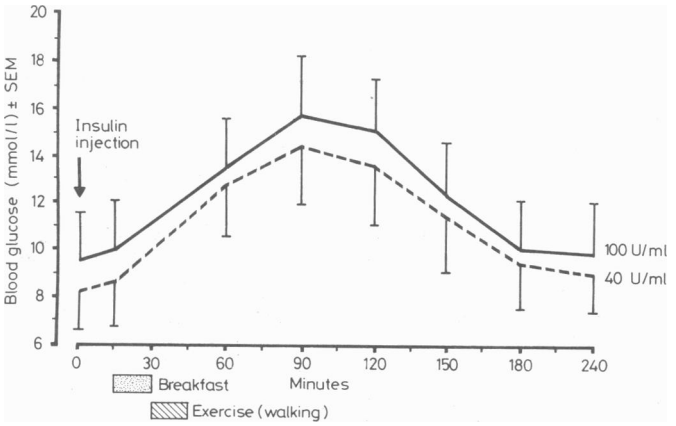
On 1 March a two year programme began in the United Kingdom of phased transfer of all diabetics requiring insulin to insulin in a strength of 100 units/ml. Factors affecting the absorption of insulin from subcutaneous tissues include site of injection,[1] exercise,[2] dose,[3] strength of the insulin, volume, and the patient's age.[4] We examined the effect of giving identical doses of soluble insulin in strengths of U-40 (40 units/ml) and U-100 (100 units/ml).

Patients, methods, and results

Eight diabetic children aged 7-15 years were given on two successive mornings in hospital exactly comparable subcutaneous injections into the thigh of either U-40 or U-100 neutral insulin (Actrapid MC) followed by identical breakfasts and exercise. The previous evening they had received their normal dose of a combination of soluble and intermediate acting insulins. To avoid rapid hypoglycaemia the dose of insulin given was roughly two thirds of the usual total morning dose, the longer acting insulin being omitted. In four cases U-40 was given on the first morning followed by U-100 on the second morning, and in the other four cases the order was reversed. Blood glucose was measured half hourly for up to four hours after breakfast and the serum insulin concentration estimated on three occasions in each child. Samples were taken through an indwelling butterfly needle to avoid repeated venepuncture. Approval of the ethical committee was obtained.

Two patients were excluded from the study because of practical difficulties. Free insulin concentrations rose from a mean basal value of 30·5±SD 23·4 mU/l in the subjects given U-40 insulin to a peak at 90 minutes of 58·8±28·3 mU/l. Patients' response to the same dose of U-100 insulin was a rise from a mean of 22·3±14·4 mU/l to 49·0±16·1 mU/l. Thus the increment in free insulin values was a mean of 28·3±14·0 mU/l after U-40 insulin and 26·7±24·0 mU/l after U-100 insulin.

The closely similar increments in free insulin concentrations after injections of U-40 and U-100 were reflected in the parallel rise and fall of the blood glucose concentrations (figure). The figure was constructed from the mean blood glucose values of six patients at specific times after the insulin injections. Thus the concentrations rose from a mean of 8·1 mmol/l (146 mg/100 ml) to a peak of 14·6 mmol/l (263 mg/100 ml) at 90 minutes after U-40 insulin. The comparative figures for U-100 insulin showed a rise from 9·6 mmol/l (173 mg/100 ml) to a peak of 16·0 mmol/l (288 mg/100 ml) at 90 minutes. The fasting blood glucose value on the second morning in hospital tended to be higher than on the first morning, which explained the discrepancy in fasting values between the two curves. Large increments in blood glucose values reflected the use of suboptimal doses of soluble insulin in this study.



Mean and range of changes in blood glucose concentrations in six insulin dependent diabetics after suboptimal doses of U-40 and U-100 insulin.

Conversion: SI to traditional units—Blood glucose: 1 mmol/l≈18 mg/100 ml.

Comment

Binder[4] showed that absorption of insulin is decreased by increasing concentration and increasing volume of injected insulin. In changing from U-40 to U-100 insulin in identical doses these two factors appear to be balanced—that is, we are giving a smaller volume of an insulin of greater strength. These variations in absorption may be mediated by effects on subcutaneous blood flow, insulin having a vasoconstrictive effect.[5]

This study showed no apparent practical difference between the absorption and effects of U-40 and U-100 insulin when given as carefully controlled single injections. Further repeated dose studies during field trials with the new glass U-100 syringes confirmed this.

From these findings the change to U-100 insulin should present few problems from the standpoint of insulin action. Nevertheless, the use of glass syringes, which have a significant dead space, the difficulties of measuring very small doses of highly concentrated insulin, and the variable quality of injection techniques and injection sites are other factors which will require consideration when patients (particularly the very young and very old) change over from U-40 to U-100 insulin.

We thank Mrs C Owens, of the department of biochemistry at Bristol Maternity Hospital, for the insulin measurements.

[1] Koivisto VA, Felig P. Alterations in insulin absorption and in blood glucose control associated with varying insulin injection sites in diabetic patients. *Ann Intern Med* 1980;**92**:59-61.
[2] Koivisto VA, Felig P. Effects of leg exercise on insulin absorption in diabetic patients. *N Engl J Med* 1978;**298**:77-83.
[3] Lauritzen T, Pramming S, Gale EAM, Deckert T, Binder C. Absorption of isophane (NPH) insulin and its clinical implications. *Br Med J* 1982; **285**:159-62.
[4] Binder C. *Absorption of injected insulin.* Copenhagen: Munksgaard, 1969.
[5] Williams G, Clark AJL, Cook E, Bowcock S, Pickup JC, Keen H. Local changes in subcutaneous blood flow around insulin injection sites measured by photoelectric plethysmography. *Diabetologia* 1981;**21**:516. (Abstract.)

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Predominance of IgG3 subclass in primary biliary cirrhosis

Mitochondrial antibodies are present in high titre in over 90% of patients with primary biliary cirrhosis[1] and are largely of the IgG class, although they may also occur in the IgM class. They are directed against a lipoprotein moiety of the mitochondrial membrane and readily fix complement in the presence of the antigen. Increased serum concentrations of immunoglobulins are common in this condition, although the IgM class is principally affected.

Using monoclonal mouse antihuman IgG subclass antibodies, we have studied the subclass distribution of mitochondrial antibodies and made a quantitative estimation of total IgG subclasses in patients with primary biliary cirrhosis.

Materials, methods, and results

The mouse monoclonal antibodies had defined specificities[2] to each of the four human IgG subclasses as well as to a common IgG Fc determinant (8a4). They were used as ascitic fluid (diluted in phosphate buffered saline) in two layer indirect immunofluorescence tests for antimitochondrial antibodies on fresh frozen sections of rat kidney and stomach. Human sera diluted one in 10 in phosphate buffered saline were added to the sections followed after washing by a predetermined appropriate dilution of the monoclonal antibody, followed by fluorescein isothiocyanate conjugated sheep antimouse IgG, rendered specific by absorption with insolubilised

Human immunoglobulin subclasses of antimitochondrial antibodies and total serum immunoglobulin and IgG subclass† values in patients with primary biliary cirrhosis*

Case No	Sex	Age	IgG Subclass				Antiserum 8a4 (anti-IgG Fc)	IgG	IgA	IgM	IgG1	IgG2	IgG3	IgG4
			IgG1	IgG2	IgG3	IgG4								
1	F	51	+	–	W	–	+ +	12·00	1·20	2·95	125	110	190	<10
2	F	59	–	–	+	–	+ +	15·05	2·85	2·60	210	225	600	70
3	F	60	+	W	+ +	–	+ +	19·95	1·95	9·10	350	225	1400	95
4	F	52	–	+	+ +	+	+ +	15·80	3·00	5·35	100	120	1050	75
5	F	30	–	+	+ + +	–	+ + +	19·05	3·45	2·20	190	225	1050	25
6	F	51	–	–	+ +	–	+ +	18·30	4·10	9·00	45	145	1600	10
7	M	62	–	–	+ +	–	+ +	ND						
8	F	80	–	W	W	–	+	16·25	4·60	2·35	260	75	775	160
9	F	59	–	–	+ +	–	+ +	ND						
10	F	53	–	W	W	–	W	35·85	3·80	3·13	600	250	900	200
11	F	63	–	–	+	–	+	11·85	2·10	1·40	80	200	160	200
12	M	68	–	W	+	–	+ +	12·90	1·25	2·70	125	160	800	10
13	M	65	–	+ + +	+	+	+ + +	15·00	1·95	3·05	160	380	1250	10
14	F	76	–	–	+ + +	–	+ + + +	23·05	2·05	2·80	350	120	1650	125
15	F	52	W	+	+ +	W	+ +	ND						
16	F	60	+	+	+ +	+	+ +	23·20	2·00	10·40	260	240	1350	90
17	F	60	W	+	+ +	+	+ + +	18·45	3·30	7·65	175	150	950	125
Normal range (mean)								6·00–16·00	0·75–4·00	M: 25–200 F: 50–240	72–128	58–154	60–150	35–200

W = "Weak" positive.
ND = Not done.

*Serum immunoglobulin concentrations expressed as g/l.
†Serum IgG subclass values expressed as % of standard pool.

human serum. Fluorescence was scored as either negative (–), weak positive (W), or definite positive (+ to + + +).

Serum immunoglobulin concentrations were measured by automated laser nephelometry. IgG subclasses were measured by the single radial diffusion test. Sera from 17 patients (14 women) with the clinical and biochemical features of primary biliary cirrhosis were referred from physicians in the West Midlands. All had a positive, classic mitochondrial antibody on routine laboratory testing, with clinical and laboratory findings consistent with the diagnosis.

The table gives the results in the sera from the 17 patients. Antimitochondrial antibodies occurred in all four subclasses. IgG3 was the most consistent and appeared in all 17 patients, IgG2 in 10 patients, while IgG1 and IgG4 antibodies were each found in only five patients.

The IgG3 antibodies also appeared to be the predominant subclass, in that in all except one of the sera they gave the most intense fluorescence; in the exception (case 13) the antibody was predominantly in the IgG2 subclass. In a further extensive study of the subclass distribution of antinuclear antibodies in patients with systemic lupus erythematosus and rheumatoid arthritis (Riggioni and Thompson, unpublished observations) this subclass restriction was not observed. The serum IgM and IgG concentrations were significantly increased in the patients ($p < 0.001$) as compared with the controls. Within the IgG subclasses IgG3 was greatly increased ($p < 0.001$). IgG1 and IgG2 were also significantly increased ($p < 0.01$) but to a less degree. There was no significant difference in IgG4 values.

Comment

In analysing IgG antibody responses several IgG subclass restrictions have been described both in animal models and in man[3 4] and changes in the distribution of IgG subclasses have also been reported in disease.

Antimitochondrial antibody, which is a hallmark of primary biliary cirrhosis, was found predominantly in the IgG3 subclass, though in many patients weaker activity in other subclasses was also present. Since IgG3, which fixes complement readily, represents only about 8% of normal IgG this is a significant restriction in subclass expression. A restriction to this subclass has not previously been described for autoantibodies. While the role and significance of antimitochondrial antibodies in primary biliary cirrhosis is unknown, their strong association with this condition at least suggests that the aetiological factors which initiate the disease are similar to those that result in the formation of these particular antibodies.

Restriction of antibody responses to IgG3 has been reported in certain viral infections.[5] Viruses have been implicated as aetiological factors in many autoimmune conditions, and our finding possibly provides some insight into the aetiology of the disease.

We are grateful to physicians in the West Midlands for supplying sera from their patients. We are also grateful to several members of the department of immunology, Birmingham University Medical School, to Drs N R Ling and R Jefferis for supplying the monoclonal antisera, to Dr D Catty for help in preparing the fluorescein isothiocyanate labelled sheep antimouse IgG, and to Miss J Lowe for advice on the subclass measurements.

[1] Doniach D. Autoimmunity in liver disease. In: Schwartz RS, ed. *Progress in clinical immunology. I.* New York: Grune and Stratton, 1972:45-70.
[2] Lowe J, Bird P, Hardie D, Jefferis R, Ling NR. Monoclonal antibodies to determinants on human gamma chains: properties of antibodies showing subclass restriction or subclass specificity. *Immunology* 1982;**47**:1-11.
[3] van der Gienssen M, Groenboer-Kempers O. The subclasses of human IgG antibodies against tetanus toxoid. *Clin Exp Immunol* 1976;**25**:117-21.
[4] Devey ME, Voak D. A critical study of the IgG subclasses of Rh-anti D antibodies formed in pregnancy and in immunised volunteers. *Immunology* 1974;**27**:1073-8.
[5] Beck DE. Distribution of virus antibody activity among human IgG subclasses. *Clin Exp Immunol* 1981;**43**:626-32.

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Effect of 1050 mg fluphenazine decanoate given intramuscularly over six days

The standard dosage of intramuscular fluphenazine decanoate for schizophrenia is usually below 100 mg a week and seldom exceeds a few hundred mg a week even in exceptional cases. We report a case of accidental overdosage, with more than 1g being given over several days.

Case report

A 24 year old Chinese woman had been diagnosed as a case of childhood psychosis, schizophreniform type, at the age of 9 and had been receiving institutional care at this hospital since the age of 18. During the first four years of her stay she had been given various maintenance regimens of phenothiazine but without effect and remained childish and self absorbed, was ambivalent about going home, and showed little interest in ward activities and occupational therapy. From the age of 22 fluphenazine decanoate 50 mg every four weeks had been added and there was a small improvement.

In February 1982 she suffered two grand mal fits and was transferred to Queen Elizabeth Hospital for investigation. During her stay there her treatment was continued except that in error the fluphenazine was given every four hours instead of every four weeks. The error was discovered on the sixth day, after 21 injections (1050 mg). She appeared to have no ill effects and resuscitative measures were not required. She had no more convulsions and was transferred back to this hospital three days later.

On her return she was in the same childish but cheerful state. At the beginning of the third week after overdosage, however, hypothermia and tachycardia were noted, and one week later features of parkinsonism appeared. Axillary temperatures ranged from 35·6° to 36·7° and the heart rate from 90 to 120 beats/min. She had immobile facies and was salivating and showed a rigid gait. These effects lasted one month, during which she was