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Rapid communication

Continuous subcutaneous insulin infusion does not induce a significant acute phase response of serum amyloid A protein

J. J. Bending[1], J. C. Pickup[2], I. F. Rowe[3], R. Gallimore[3], G. Tennent[3], H. Keen[1] and M. B. Pepys[3]

[1]Unit for Metabolic Medicine and [2]Department of Chemical Pathology, Guy's Hospital Medical School and [3]MRC Acute Phase Protein Research Group, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK

Summary. In a study of 23 matched pairs of Type 1 (insulin-dependent) diabetic patients receiving continuous subcutaneous insulin infusion or conventional insulin injection therapy respectively, there were no significant differences in serum levels of the acute phase proteins, serum amyloid A and C-reactive protein. These results do not support the suggestion that continuous subcutaneous insulin infusion stimulates serum amyloid A production or that it carries a risk of inducing reactive systemic amyloidosis.

Key words: Continuous subcutaneous insulin infusion, amyloidosis, C-reactive protein, acute phase response.

It has been suggested lately that a possible long-term hazard of continuous subcutaneous infusion (CSII) may be the development of reactive systemic amyloidosis. Beagle dogs receiving continuous intravenous insulin infusion were reported to have systemic amyloid deposits at autopsy [1]. In a recent study [2], the concentration of serum amyloid A protein, the putative precursor of amyloid A fibrils, was measured in diabetic patients treated by CSII, patients receiving conventional insulin injections and in one individual deliberately given insulin which was thought to be aggregated. The results were interpreted as showing that infused or injected aggregated insulin were potent stimuli for the acute phase response.

Table 1. Clinical features and acute phase responses to two different methods of insulin administration

	Treatment		*p*
	CSII	Injection therapy	
	(*n* = 23)	(*n* = 23)	
Sex (M:F)	15:8	15:8	
Mean age of patients (years)	38.5 (17–64)	39.3 (16–63)	NS
Mean duration of diabetes (years)	19.3 (2–33)	19.7 (3–39)	NS
Mean daily insulin dose (units/kg)	0.6 (0.3–1.4)	0.7 (0.36–1.17)	NS
Median Serum amyloid A (U/l)[a]	< 80 (< 80–266)	< 80 (< 80–> 5,000)	NS
Median C-reactive protein (mg/l)[b]	< 1 (< 1–2)	< 1 (< 1–43)	NS
Median Erythrocyte sedimentation rate (mm/first h)	5 (1–60)	5 (1–63)	NS

Ranges given in parentheses
[a] Normal = < 100 U/l; [b] Normal = < 10 mg/l

Other small scale studies [3, 4], argue against a risk of amyloidosis during pump treatment so that, in view of the serious consequences of systemic amyloidosis, and the increasing use of CSII as a routine treatment for Type 1 diabetes and in clinical trials concerning the management and causes of diabetic complications, it is important to resolve this issue.

We report here on the concentration of serum amyloid A and C-reactive protein in 23 pairs of Type 1 diabetic patients matched precisely for age, sex, duration of diabetes and presence and severity of complications. One member of the pair was receiving insulin by CSII and the other by conventional subcutaneous injection therapy. All patients were submitted to standardised enquiry and full physical examination at the time of blood sampling.

Subjects and methods

Subjects

Twenty-three diabetic patients (15 males, 8 females), treated by CSII and who were consecutive attenders at a review clinic, were recruited for the study. The clinical characteristics of the patients studied are shown in Table 1. The group included nine patients with diabetic nephropathy, three with intermittent proteinuria and six with persistent proteinuria (i.e. positive testing with Albustix; Ames-Miles, Stoke

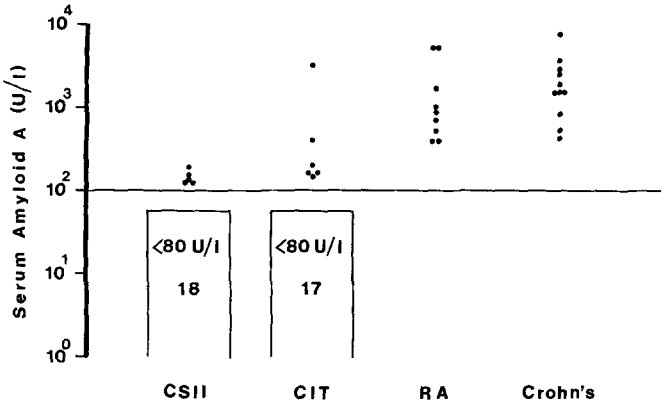

Fig. 1. Serum amyloid A concentrations in 23 patients treated by continuous subcutaneous insulin infusion (CSII) and 23 patients treated by conventional-injection therapy (CIT) compared with concentrations found in nine patients with severe rheumatoid arthritis (RA) and 11 patients with severe Crohn's disease (Crohn's) [10]. The horizontal line at a serum amyloid A concentration of 100 U/l represents the upper limit of normal. Values < 80 U/l lie below the limits of detection of the assay

Poges, Bucks, UK). The patients were closely matched for age, sex, duration of diabetes and degree and severity of complications with 23 patients treated by conventional injection therapy. Selection of the control group was from patients routinely attending the diabetic outpatient clinic who fulfilled the detailed matching criteria.

Methods

All patients in both treatment groups were systematically questioned according to a pre-determined protocol which included a detailed systems review, and a record of alcohol and smoking habits (long-term and recent) and drug history. For each patient we noted the type, strength and expiry date of the insulin used. For CSII-treated patients, types of delivery cannulae and infusion techniques were detailed, including infusion sites, frequency of changing and time of last change of both site and cannula. Patients were questioned for any history of redness, bleeding, bruising, pain or lumps noted at the infusion or injection sites. For patients treated by conventional insulin injections, the number, type and dose of insulin injections per day and the time since last injection were recorded. All patients then underwent a physical examination which included the measurement of oral temperature and an examination of injection or infusion sites.

Insulin was infused in all CSII-treated patients into the subcutaneous tissue of the anterior abdominal wall via 25 G winged-needle cannulae (Butterfly, Abbott Laboratories, Queenborough, Kent, UK or Steritex, Espergaerde, Denmark) as described previously [5]. Insulins used were either 40 U/ml porcine Actrapid (Novo Laboratories, Basingstoke, Hants, UK), diluted as necessary to between 10 and 30 U/ml for the Mill Hill Infuser model 1001 HM (Muirhead Medical, London, UK) or Velosulin (100 U/ml) in the special pre-filled syringes used with the Nordisk Infuser (Nordisk Gentofte, Denmark). Conventionally-treated patients, with one exception, received two or more injections a day using combinations of short-, intermediate- or long-acting insulins. The one exception received one daily injection of a mixture of Actrapid and Monotard insulins (Novo) before breakfast.

Venous blood samples were allowed to clot at room temperature for up to 6 h, and serum was then separated and stored at −20 °C prior to assay. All assays were performed in ignorance of the treatment received in a single batch at the end of the study. Serum C-reactive protein levels were measured by electroimmunoassay calibrated with isolated pure C-reactive protein as previously described [6] and serum amyloid A concentrations by single radial immunodiffusion in agarose gel [7], using a monospecific rabbit antiserum to human serum amyloid A [8]. The assay, which had a coefficient of variation of < 10%, was calibrated with an acute phase serum containing 10,000 U/l of serum amyloid A, using an immunoradiometric technique [8]. The normal range in serum from 100 healthy subjects was < 1 to 100 U/l [8]. Erythrocyte sedimentation rate was measured by the Westergren method [9].

Non-parametric comparisons of the groups were made by the Wilcoxon rank test.

Results

The values for serum amyloid A, C-reactive protein and erythrocyte sedimentation rate in the CSII- and conventionally injected groups are shown Table 1. The individual values of serum amyloid A concentration for all patients are shown in Figure 1, which also shows, for comparison, the levels of serum amyloid A found during an earlier study [10] in nine patients with severe rheumatoid arthritis and in 11 patients with severe Crohn's disease. Five patients receiving CSII had serum amyloid A levels above those in the nondiabetic control subjects though none had abnormal C-reactive protein levels; in the conventionally-treated group, six patients had raised serum amyloid A concentrations and three raised C-reactive protein levels. The two diabetic groups did not differ significantly from each other.

Only two individuals with raised serum amyloid A had levels which were more than trivially elevated, and both of these (611 and > 5,000 U/l, respectively) were in the conventionally-injected group. The former was a woman of 52 years with diabetes of 18 years' duration without complications other than moderate non-proliferative retinopathy. She had a C-reactive protein concentration of 40 mg/l but was apyrexial with a total white cell count of 8.8×10^9/l; there was no clinical evidence of any active intercurrent pathological process. The second individual was a 53-year-old man with diabetes of 24 years' duration complicated by proliferative retinopathy, persistent proteinuria, reduced glomerular filtration rate and autonomic and peripheral neuropathy. He had a C-reactive protein concentration of 39 mg/l, and despite being apyrexial, complained of a non-productive cough and had a neutrophil leucocytosis (total white count 12.9×10^9/l, neutrophils 8.6×10^9/l) and an erythrocyte sedimentation rate of 49 mm/first h. No patient in either group had an oral temperature exceeding 37.0 °C. Inspection of infusion sites in CSII-treated patients revealed the presence of no more than minimal erythema associated with the needle insertion in any patient. Injection sites in conventional-injection-treated patients showed no evidence of local inflammation.

Repeat measurements were performed on two patients before and after institution of CSII treatment. In one, the C-reactive protein concentration on days 0 and 14 was < 1 and 2 mg/l, respectively and the serum amyloid A level was 175 U/l on both occasions. In the other

patient, both the C-reactive protein and serum amyloid A values were normal (< 1 mg/l and < 80 U/l, respectively) both on days 0 and 9.

Discussion

The present results do not support the idea that CSII is an acute phase stimulus per se, nor indeed is conventional insulin injection. The few minor elevations of serum amyloid A and C-reactive protein seen in the present study probably reflect intercurrent events unrelated to the type of insulin or mode of administration.

Our findings extend the recent report by Deckert and Lauritzen [11] although they are not in agreement with the conclusions of Brownlee et al. [2]. However, the application of a non-parametric ranking test (Mann-Whitney U-test) to the serum amyloid A values reported by Brownlee at al. does not reveal a statistically significant difference between their CSII- and conventionally-treated groups. Since amyloid A in its native state in serum is an apolipoprotein of high density lipoprotein, there are many problems associated with its immunoassay, particularly the question of standardisation. The use of denatured amyloid A protein in some assays [2], which represents only a part of the whole serum amyloid A polypeptide, may not therefore reflect the absolute quantity of serum amyloid A present. More important, however, is the need to know in relative terms whether a given acute phase response is substantial or not. There is much evidence to support the view that major and sustained elevation of serum amyloid A levels is a necessary, though not exclusive, condition for development of systemic amyloidosis. Diseases, such as ulcerative colitis and systemic lupus erythematosus, in which only modest values for serum amyloid A are seen, are complicated by amyloid only exceptionally [10]. This contrasts sharply with the situation in rheumatoid arthritis, Still's disease and Crohn's disease [10]. In the present study, most serum amyloid A values above the upper limit of normal (100 U/l) were raised only marginally, whilst the one individual with a massive elevation, who was not on CSII, had good objective evidence of an intercurrent infection. No difference in serum amyloid A levels was apparent between those patients treated by CSII who received infusions of diluted strengths of 10–30 U/ml and those who received infusions of concentrated insulin at a strength of 100 U/ml.

We therefore conclude, both from our own and other published results, that the available evidence does not implicate CSII as an inevitable or potent stimulus for the acute phase response in general or of serum amyloid A in particular. Although caution should continue to be exercised in the evaluation of this relatively new technique, there seems no reason at present for suspecting that CSII might carry a risk of inducing systemic amyloidosis.

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Dr. J. C. Pickup
Department of Chemical Pathology
Guy's Hospital Medical School
London SE1 9RT
UK.