Effects of insulin antibodies on bioavailability of insulin: preliminary studies using ^{123}I -insulin in patients with insulin-dependent diabetes

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Summary. Bovine insulin was labelled with carrier-free Na ^{123}I and the species labelled on the A14 tyrosyl residue was purified by reverse phase high pressure liquid chromatography. After sterilization by filtration through a 0.22 μm millipore filter, the labelled hormone (^{123}I -insulin) was injected intravenously into normal volunteers and into two insulin-immunized insulin-dependent diabetic patients. Patient 1 was treated with 74 U insulin/day, whereas, despite daily insulin doses > 100 U, patient 2 remained hyperglycaemic and continued to lose weight. Plasma insulin binding capacity was 10 U/l in patient 1 and > 20 U/l in patient 2. In both diabetic patients, heart activity (i.e. blood pool) decreased more slowly than in control patients, an observation consistent with the reduction of plasma insulin clearance rate in immunized pa-

tients. Kidney activity was decreased in patient 1 and undetectable in patient 2, suggesting that most of the circulating ^{123}I -insulin was bound to antibodies and not filtrable. Finally, in both patients, the profile of liver radioactivity was markedly altered. Maximum liver activity was delayed and the liver image persisted for > 45 min. According to analysis of the time activity profile in various organs, we consider that specific antibodies act predominantly either as 'carrier-proteins' retarding the action of soluble insulin as in patient 1 or as insulin 'scavengers', the immune complexes being cleared by the reticulo-endothelial system as in patient 2.

Key words: ^{123}I -insulin, anti-insulin antibodies, insulin-dependent diabetes.

We have demonstrated that ^{123}I -monoiodo A14 tyrosyl insulin purified by high pressure reverse phase liquid chromatography approximates to the ideal tracer for studies of insulin in vivo (theoretical value of the sp. act. 41 mCi/ μg ; actual value probably four to ten times lower). When produced by the $^{127}\text{I}(\text{p}, 5\text{n})$ reaction, ^{123}I is devoid of the radiotoxic isotope ^{124}I and is only slightly contaminated with < 0.2% ^{125}I [2]. ^{123}I decays by emission of a γ photon of 159 KeV, an X photon of about 30 KeV and no β particles. These properties, together with its short physical half-life (13.3 h), account for its low radiotoxicity in man and its high quality as an 'imaging' isotope in nuclear medicine.

When ^{123}I -insulin is injected intravenously into normal rats and into man, it is rapidly concentrated by the liver and kidneys by different mechanisms. In the liver, ^{123}I -insulin binds to specific receptors on the hepatocytes. Animal studies have demonstrated this binding to be saturable, specific and blocked by antibodies to the insulin receptor [1, 3]. Furthermore, autoradiographic studies performed with ^{125}I -insulin have demonstrated binding to the hepatocyte membrane [4]. In contrast, ^{123}I -insulin uptake by the kidneys occurs by a non-re-

ceptor mediated mechanism consisting of filtration through the glomeruli and reabsorption by the proximal tubules [5].

In normal human volunteers, liver radioactivity reaches a maximum 6–8 min after ^{123}I -insulin injection and then rapidly decreases as ^{123}I -insulin is degraded, residual ^{123}I -iodotyrosine is dehalogenated and free ^{123}I is released into the plasma (Fig. 1). Normally, the hepatic image disappears 20 min after ^{123}I -insulin injection [1].

In the present study, we report preliminary studies of the biodistribution of ^{123}I -insulin in insulin-immunized diabetic patients.

Patients and methods

Patient 1 was a Caucasoid female, aged 28 years, with Type 1 (insulin-dependent) diabetes diagnosed at 17 years of age. Her diabetes was 'brittle', requiring three insulin injections/day at doses adjusted by the patient according to the results of blood glucose home monitoring. At the time of the study, she was receiving daily a total of 42 U insulin suspension (Novolente; 70% bovine, 30% porcine) and 32 U soluble insulin (Actrapid, porcine). Plasma anti-insulin antibodies had a binding capacity of 10 U/l.

Fig. 1. Time activity curves of liver (●) and heart (▲) of a normal volunteer. The matrix size of the images recorded by the computer was 64×64 picture elements (or pixels) and activity is expressed as counts per pixel per 30 s

Patient 2 was a Caucasoid male, aged 65 years, treated by oral antidiabetic drugs until 1982, and then by increasing doses of insulin suspension (Novolente). Despite 120 U of insulin daily, glycosuria was massive, fasting blood glucose was often >22 mmol/l and he continued to lose weight. The insulin binding capacity of the plasma was >20 U/l.

^{123}I -insulin was prepared as described previously by iodinating bovine insulin with carrier-free $\text{Na } ^{123}\text{I}$. The species labelled on A14 was isolated by high pressure liquid chromatography and sterilized by ultra-filtration through a $0.22 \mu\text{m}$ millipore filtre [1].

The patients lay supine under the detector of a gamma camera connected to a computer and received approximately 1 mCi ^{123}I -insulin at time zero via an antecubital vein. Analogue images were taken at selected times. After 45 min observation period, regions of interest were defined over the heart, liver, spleen and kidneys and time-activity curves were obtained.

Results

In contrast with normal man, the scintigraphic profile of patient 1 showed that the heart (i.e. blood pool) remained visible throughout the 45-min investigation period. Liver activity reached a maximum at around 10 min and decreased slowly, the liver being still visible after 45 min. The kidneys and the spleen were detectable, although much less radioactive than the liver.

Time-activity curves of the heart, liver, and spleen of patient 2 are shown in Figure 2. The heart activity (i.e. blood pool) remained abnormally high throughout the 45-min observation period. Liver activity reached a maximum after 20 min compared with 6–8 min in normal subjects and remained at that level during the rest of the study. The spleen was barely detectable. The kidneys were not visible at all.

Fig. 2. Time activity-curves of the liver (●), heart (▲) and spleen (■) of patient 2. Note that for this patient, the time scale was extended to 45 min. The matrix size was the same as in Figure 1, but other recording conditions were different so that absolute values of the ordinates of Figures 1 and 2 are not comparable

Discussion

Virtually all insulin-treated diabetic patients become immunized against insulin [6]. Yet little is known about the effect of anti-insulin antibodies on the bioavailability of therapeutically administered insulin [7–10]. Bollinger et al. found that the plasma clearance rate of ^{131}I -insulin was decreased in insulin-immunized patients [11]. A similar conclusion may be reached from the two patients in this study, in whom heart radioactivity (i.e. blood pool) decreased much more slowly than in normal volunteers. Insulin antibodies are presumed to form immune complexes with ^{123}I -insulin. These complexes are, in turn, in equilibrium with free ^{123}I -insulin. The free ^{123}I -insulin appears as radioactivity in the kidneys. The free hormone also binds to hepatic insulin receptors and is metabolised by hepatocytes (patient 1). Continuous removal of free hormone by the insulin receptors and the kidneys will displace the equilibrium towards the dissociation of the circulating immune complexes. Insulin antibodies may thus be regarded as 'carrier proteins', similar to cortisol binding globulin for cortisol or thyroxine binding globulin for thyroxine and so retard the action of plasma insulin. That antibodies may play such a role has been demonstrated by the delayed appearance of acidosis in insulin-immunized patients after acute withdrawal of insulin [12].

In patient 2, the biodistribution of insulin is even more severely altered. The kidneys did not take up visible amounts of radioactivity. We interpret this to mean that the plasma concentration of free ^{123}I -insulin was negligible. Yet the liver of this patient took up a significant amount of radioactivity and we postulate that it in-

teracted with the immune complexes and not with the free hormone. At the present time, there is no convincing evidence that insulin-antibody complexes may bind to insulin receptors. The alternative explanation for hepatic uptake of radioactivity would therefore be that the immune complexes are taken up by the Kupffer cells. This mechanism would be quite comparable with that observed in passively immunized rats with appropriate doses of guinea pig insulin antiserum. In this case, intravenously injected ^{125}I -insulin is taken up by the liver entirely and moreover, as in patient 2, after reaching its maximum, liver radioactivity remains at a plateau for at least 20 min. Autoradiographic studies of the liver of passively immunized rats injected with ^{125}I -insulin demonstrated that silver grains were concentrated in the Kupffer cells [13]. Thus in patient 2, by analogy with animal studies, antibodies would act as insulin scavengers. Much less of the antibody bound ^{125}I -insulin is bioavailable, which may explain the persistent and marked hyperglycaemia in this patient, despite high daily doses of insulin. The mechanism of insulin scavenging may also be operative in patient 1, although to a much lesser extent.

The effect of insulin antibodies in the circulation of insulin-treated patients on insulin bioavailability cannot be predicted at the present time in any given patient, despite measurement of insulin-binding capacity. As reported here, the ^{125}I -insulin test, a simple procedure with low radiotoxicity, gives a direct estimate of insulin bioavailability in vivo. Further studies, in conjunction with more precise identity the various constituents, e.g. the antibody class, avidity, complement binding and epitopes on insulin that interact with the antibodies should help to elucidate the mechanisms involved.

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