In Vivo Chloroquine-induced Inhibition of Insulin Degradation in a Diabetic Patient with Severe Insulin Resistance

BRUCE R. BLAZAR, CHESTER B. WHITLEY, ABBAS E. KITABCHI, MICHAEL Y. TSAI, JULIO SANTIAGO, NEIL WHITE, FRANKIE B. STENTZ, AND DAVID M. BROWN

SUMMARY

We report on a 26-yr-old patient with an 11-yr history of insulin-dependent diabetes mellitus who exhibited insulin resistance with a requirement of up to 15,000 U of intravenous (i.v.) insulin/day. Attempts to diminish her insulin requirement by administration of sulfated insulin or Trasylol were unsuccessful, with the patient remaining resistant to subcutaneous (s.c.) and i.v. administration of pure pork insulin. Chloroquine phosphate therapy (500 mg twice a day) resulted in a decreased requirement for i.v. insulin (700 U/day as compared with the pretreatment requirement of 8400 U/day). Accelerated insulin degradation in s.c. fat tissue of the patient before treatment with chloroquine was demonstrated. This activity was decreased by 64% during chloroquine therapy. Inhibition of insulin degrading activity (IDA) during chloroquine therapy was associated with reductions in the leukocyte lysosomal enzymes α-galactosidase and hexosaminidase-A but not hexosaminidase-B and β-glucuronidase. This study constitutes the first reported use of chloroquine for treatment of insulin resistance as a result of accelerated insulin degradation, and it provides evidence of the effectiveness of this agent in this rare condition.

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Insulin resistance has been attributed to several hormone and end-organ factors[1] including altered insulin metabolism.[2] Increased rates of insulin degradation have been found in several patients who are resistant to massive doses of s.c. insulin but who respond normally to smaller doses of i.v. insulin.[3–7]

Herein is presented a patient who was resistant to massive doses of insulin administered both s.c. and i.v., and whose adipose tissue exhibited excessive insulin degradation. Based on the inhibition of insulin degradation by protease inhibitors in vitro, we initiated a series of therapeutic trials that demonstrated beneficial effects of chloroquine but not of Trasylol. The in vitro effects of chloroquine correlated with in vitro insulin degrading activity of s.c. tissue in the presence and absence of chloroquine.

CASE REPORT

N.B., a 26-yr-old white woman, had had insulin-dependent diabetes mellitus since the age of 9 yr. During the subsequent 15 yr, she was treated with 1 or 2 s.c. injections of NPH and regular insulin in total daily doses ranging from 36 to 75 U. Between the ages of 9 and 15 yr, she was hospitalized 11 times for treatment of ketoacidosis. At the age of 18 yr, she was noted to have progressive retinopathy with bilateral microaneurysms, hemorrhages, and exudates, and had a creatinine clearance of 59 ml/min/1.73 m^2. At the age of 21 yr, she developed bilateral lower leg pain attributed to diabetic neuropathy and had hypertension, 140/100 mm Hg. A kidney biopsy at that time was consistent with diffuse glomerulosclerosis. Her glycosylated hemoglobin was 18.7% (normal 5.5–8.5%). In the 3 yr before the present illness, she had three additional episodes of ketoacidosis, each of which responded readily to standard i.v. and s.c. insulin regimens. In the year before the present illness, she received a total daily s.c. insulin dose of 2.7 U/kg, although she required nine hospitalizations in an attempt to achieve moderate metabolic control at which time her glycosylated hemoglobin was 19.3%. Her retinopathy and vision progressively deteriorated, necessitating laser treatment of the right retina.

The patient was noted to be unresponsive to 5 U/kg/day of s.c. regular insulin but was sensitive to continuous i.v. regular insulin at rates of 0.01–0.1 U/kg/h. Several insulin counterregulatory hormone measurements revealed only

From the Departments of Pediatrics and Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota (B.R.B., C.B.W., M.Y.T., D.M.B.); the Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri (J.S., N.W.); and the Departments of Medicine and Biochemistry, University of Tennessee Center for the Health Sciences, Memphis, Tennessee (A.E.K., F.B.S.).

Address reprint requests to David M. Brown, Ph.D., M.D., Departments of Pediatrics and Lab. Med. Path., University of Minnesota, Mayo Box 293, Minneapolis, Minnesota 55455.

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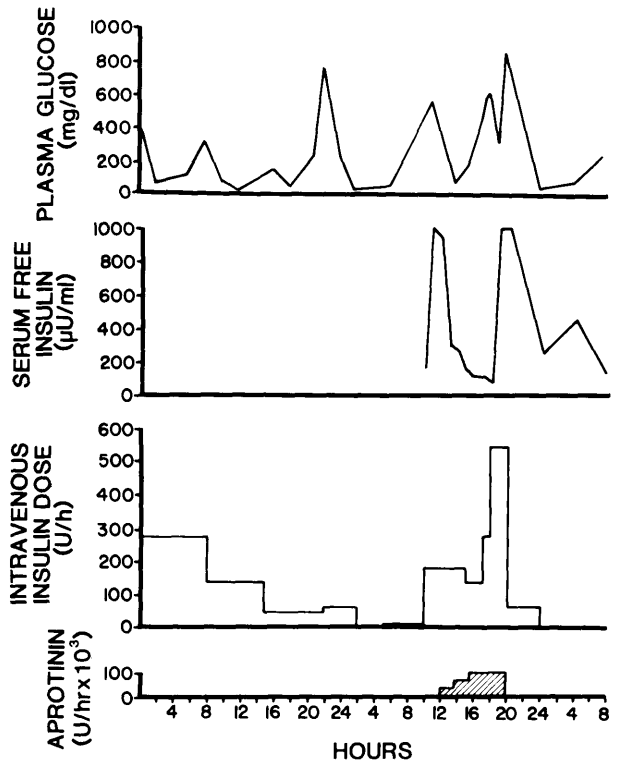

FIGURE 1. Lack of an effect of aprotinin on the effects of insulin.

mild abnormalities, including: 8 a.m. plasma cortisol levels of 32 and 34 μg/dl (corresponding plasma glucose 112 and 840 mg/dl); urinary free cortisol, 291 μg/24 h (normal 78–365); 24-h urine metanephrine, 2.5 mg/mg creatinine (normal 0.0–0.90); vanilmandelic acid, 4.7 mg/day (normal 0–6.2, corrected for age); homovanillic acid, 4.6 mg/day (normal 0.0–5.7 mg/day); plasma norepinephrine, 198 pg/ml (normal 0–30); plasma glucagon, 168 pg/ml (normal 50–200); and growth hormone, 16 ng/ml (normal 0–10). The serum-free thyroxine index was 5.5 (normal 5.0–11.5) and thyrotropin 3.5 μU/ml. Total serum insulin (0.52–2.4%) was bound to proteins (normal values: nondiabetic patients <1%; insulin-dependent diabetic subjects 90%, <15.05). No serum anti-insulin receptor antibodies (measured by Dr. C. Ronald Kahn, Joslin Clinic, Boston, Massachusetts) were detected.

Trials of s.c. monocomponent pork insulin (Eli Lilly and Company, Indianapolis, Indiana) and sulfated beef insulin (courtesy of Dr. John K. Davidson, Emory University, Atlanta, Georgia) using 3–4 consecutive doses of up to 200 U/dose at 2-h intervals failed to maintain the patient's plasma glucose <600 mg/dl. Because of the possibility of the patient having accelerated insulin degradation, a fat biopsy was performed for assay of insulin degrading activity (IDA). Incubation of both the patient's and a normal subject's sera with ^{125}I-insulin revealed no ^{125}I-insulin degradation.

An attempt was made to inhibit insulin degradation in vivo using the protease inhibitor, aprotinin (Trasylol).[6] By that time, insulin resistance had increased, requiring as much as 200 U/h of regular i.v. insulin. Despite the use of repeated (every 2 h) doses of as much aprotinin as 4–160 KIU/U of insulin s.c. or 100,000 U of aprotinin/h i.v., no change was noted in insulin requirements (Figure 1).

Free insulin levels decreased to 80 μU/ml and plasma glucose rose initially to 610 mg/dl, requiring a further increase of i.v. insulin to 540 U/h to achieve a free insulin level of >1000 μU/ml and to reduce the plasma glucose from 850 mg/dl to 40 mg/dl. Thus, the patient's satisfactory response to insulin required high levels of free insulin but had no relationship to aprotinin administration.

Metabolic conditions before chloroquine phosphate therapy. As the patient continued to require 2500–6500 U of i.v. insulin/day with continued moderate ketones in the urine, an attempt was made to combat insulin resistance by other modulators of insulin metabolism. Chloroquine phosphate, a known modulator of insulin processing,[8] was administered orally (500 mg) twice daily. The effect of the drug was monitored by measuring leukocyte lysosomal enzyme activities at regular intervals. After 2 mo of chloroquine phosphate therapy, the patient still required 80–125 U/h of insulin given by continuous i.v. infusion to maintain plasma glucose concentrations of 80–300 mg/dl. Therefore, chloroquine treatment was discontinued. Within 2 days, however, the patient's insulin requirements increased to 680 U/h i.v. and remained at that level for 24 h, during which time serum-free insulin levels were 382 and >1000 μU/ml. To assess the role of drug treatment, chloroquine phosphate therapy was reinstituted at a dose of 500 mg orally three times daily. During the next 3 days, the amount of i.v. insulin necessary to maintain plasma glucose levels 80–300 mg/dl decreased to 4–12 U/h. On the second day, the serum-free insulin level was 601 μU/ml. Four weeks after restarting chloroquine phosphate, a second fat biopsy was obtained for studying IDA. An empirical trial of dexamethasone therapy, 10 mg/m^2/day for 4 days, did not alter insulin requirements.

TABLE 1
Insulin degrading activity (IDA) in s.c. fat tissue of a diabetic patient with insulin resistance before and after chloroquine therapy

Additions to the incubation system	Before chloroquine		During chloroquine	
	IDA*	% Inhibition	IDA*	% Inhibition
None	7.2	—	2.6	—
Chloroquine (0.5 mM)	3.2	55	2.0	24
Aprotinin (1000 KIU)	6.0	17	2.1	21
Glucagon (10^{-6} M)	6.3	13	1.9	26
N-ethylmaleimide (0.01 M)	0	100	0	100

*Femtomoles of insulin degraded/min/mg protein. Values are mean of initial rate calculated from a linear curve constructed at 0, 5, 10, and 30 min of incubation assay (see text for details). IDA in fat biopsy from control lean subjects (N = 10); 1.9 ± 0.3 fmol of insulin destroyed/min/mg protein. For details of assay see MATERIALS AND METHODS.

The chloroquine phosphate therapy was discontinued and the patient was maintained on 100–250 U/h of i.v. insulin. At that time, plasma insulin antibodies remained unchanged from previous levels.

MATERIALS AND METHODS

Assay of insulin degrading activity. The method for enzyme assay was as described before[9] with the following modifications in enzyme preparation. One gram of fat tissue obtained from the anterior thigh under local anesthesia was homogenized in 2 ml of 0.33 M sucrose at 4°C in a glass homogenizer. The homogenate was then centrifuged at 100,000 × *g* for 60 min. The supernatant, which contained enzyme activity, was used throughout the studies. The incubation medium for degradation studies consisted of A_{14}-^{125}I-insulin (250–360 μCi/μg) (10^{-10} M) containing approximately 40,000 cpm/ml, plus 0.1 M Hepes buffer, pH 7.6, with 0.3% dialyzed bovine serum albumin. To 0.9 ml of the buffer containing ^{125}I-insulin, 0.1 ml of 100,000 × *g* supernatant was added and the incubation mixture was carried out in triplicate at 0, 5, 10, and 30 min at 37°C. At the end of each incubation time, 1 ml of 10% TCA was added. The reaction mixture was then centrifuged at 1800 × *g* for 15 min and the supernatant and precipitates were separated and counted in a gamma spectrometer. The percent degradation was calculated as cpm in the supernatant over cpm in the supernatant plus precipitate, and the values were then converted to femtomoles of insulin degraded. The amount of protein in the enzyme preparation was determined by the method of Lowry et al.[10] Specific activity was expressed as femtomoles of insulin degraded/min/mg protein, calculated as initial rate from the constructed curve activity of four time periods (0, 5, 10, and 30 min), which was linear with time at 37°C. Results were compared with the values obtained from adipose tissue of 10 nondiabetic subjects.

Other assays. Serum degradation of insulin was determined by the method of Misbin et al.[4]

Leukocyte α-galactosidase, hexosaminidase-A, hexosaminidase-B, and β-glucuronidase were measured by methods described previously.[11–13]

Serum insulin antibodies were determined by the method of Skom and Talmage.[14] Free insulin was determined by the method of Kuzuya et al.[15]

RESULTS

Adipose tissue insulin degradation (Table 1). Insulin degrading activity by the patient's adipose tissue in vitro before the administration of chloroquine was more than threefold greater than normal values (7.2 versus 1.9 fmol). IDA was substantially inhibited by the addition of chloroquine but was minimally affected by aprotinin and glucagon. As had been previously reported,[16] N-ethylmaleimide completely inhibited insulin degradation. Three weeks after in vivo administration of chloroquine, insulin degradation by the patient's adipose tissue was markedly decreased, whereas the in vitro addition of chloroquine had less effects as compared with the inhibition achieved by either of those agents before chloroquine therapy.

Leukocyte lysosomal enzyme activities (Figure 2). The effect of chloroquine on leukocyte lysosomal enzymes was studied after the patient had been receiving chloroquine phosphate for 5 wk. Initial activities of α-galactosidase, hexosaminidase-A, hexosaminidase-B, and β-glucuronidase were within 20% of control values. After 4 wk of chloroquine treatment, a marked decrease in α-galactosidase activity occurred, falling to 42% of control activity after 65 days and to 36% of control values after another 60 days. Three weeks after therapy was discontinued, α-galactosidase activity had returned to normal. Hexosaminidase-A activity paralleled that of α-galactosidase, reaching its lowest level (56% of control) after 65 days of chloroquine treatment. In contrast to the reduced activities of α-galactosidase and hexosaminidase-A, hexosaminidase-B activity remained within 10% of control levels, while β-glucuronidase activity increased from 158 to 207% of control during chloroquine treatment, decreasing to control activity by 3 wk after cessation of the drug.

DISCUSSION

The patient described in this report represents an example of extreme resistance to both s.c.- and i.v.-administered in-

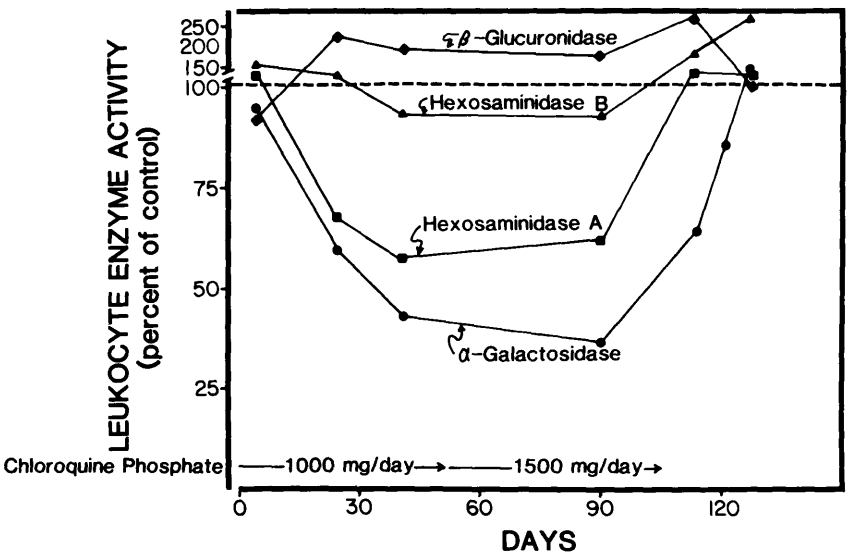

FIGURE 2. Effect of chloroquine treatment on lysosomal enzymatic activities. At the beginning of the study period, four leukocyte lysosomal enzyme activities approximated control levels (percent of mean). During the subsequent period of treatment with chloroquine, α-galactosidase was markedly reduced and hexosaminidase-A levels exhibited a parallel decline. On cessation of chloroquine administration, α-galactosidase and hexosaminidase-A activities returned to control levels.

sulin. There was no evidence of other causes of insulin resistance, such as abnormalities of growth hormone, glucagon, cortisol, or catecholamine metabolism beyond what might be expected of a patient with insulin-dependent diabetes mellitus under stress. She had normal ovarian function, no acanthosis nigricans, and no serum anti-insulin receptor antibodies. Serum insulin antibody levels were not increased. Her insulin resistance was partially ameliorated by systemic administration of chloroquine. Accelerated insulin degradation in s.c. fat tissue was partially inhibited, concomitant with the partial reversal of the insulin resistance.

Increased insulin degrading activity as a cause of postreceptor insulin resistance has been postulated since 1964,[17] but only recently have in vitro measurements of insulin degrading activity been correlated with in vivo phenomena in a limited number of cases.[2-5] Insulin degradation, as reviewed recently,[8] is achieved through three enzymic processes. Glutathione insulin transhydrogenase (GIT) is a microsomal enzyme that degrades not only insulin but also other disulfide-containing proteins by cleavage of these bonds.[18,19] Lysosomal protease also degrades insulin, but at acid pH. Insulin (neutral) protease, on the other hand, is a proteolytic enzyme that is specific for insulin[20] and glucagon degradation[21] and is located in both the particulate and soluble fractions of cell homogenates of all tissues examined.[22] More than 90% of insulin protease, however, is in the soluble fraction. Insulin protease constitutes more than 95% of all IDA in human muscle[9] and fibroblast[23] as well as in fat tissues of rats.[22] Since we used 100,000 × *g* supernatant fractionated from the fat tissue, the likelihood of GIT being present in this fraction is negligible, as the major portion of IDA in this fraction is due to insulin protease.

Recent studies of insulin degradation in human skeletal muscle suggest that the most effective inhibitors of insulin degradation are *p*-hydroxymercuribenzoate, N-ethylmaleimide, and bacitracin.[9] The lysosomotropic agent, chloroquine, also inhibits insulin degradation in muscle,[9] but more effectively in human fibroblast.[23] Although the mechanism of action of chloroquine on IDA in this patient has not been fully studied, it is tempting to postulate that chloroquine affects both neutral and acid protease activity in both s.c. fat tissue and liver. Although IDA in this patient's fat tissue was approximately three times greater than in normal fat tissue, this could not explain the tremendous difference in the insulin requirement of nonresistant and resistant diabetes. However, as this patient showed insulin resistance to both i.v. and s.c. insulin administration, it is tempting to postulate that the major changes in IDA occurred in the liver, where IDA is normally 10 times more active than in the fat tissue in nondiabetic tissues.[22] The mechanism of accelerated insulin degradation in both liver and fat tissues could partially explain such a massive insulin resistance. As chloroquine is known to inhibit both hepatic and adipose IDA in vivo,[8] it is conceivable that this agent inhibited IDA in vivo in both fat and liver with resulting partial amelioration of insulin resistance.

Increased insulin degradation in this patient was confirmed by the demonstration of increased insulin degradation by her adipose tissue in vitro, while there was no evidence of accelerated insulin degradation by her serum, emphasizing that increased insulin degradation was predominantly an intracellular process. Chloroquine, which inhibited insulin degradation in vitro by 55%, was administered in phosphate form in high oral doses usually reserved for treatment of severe arthritis. A second study of adipose tissue insulin degradation in vitro at that time showed a 64% reduction of activity and reduced effects of protease inhibitors.

To our knowledge, this is the first reported case of therapeutic use of chloroquine phosphate in insulin resistance due to accelerated insulin degrading activity. This drug may be an effective method of therapy for such rare cases.

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