Immunological and Metabolic Responses of Patients With History of Antibody-Induced Beef Insulin Resistance to Treatment With Beef, Pork, Human, and Sulfated Beef Insulin

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OBJECTIVE — We evaluated immunological and metabolic responses during therapy with beef (B), pork (P), human (H, rDNA), and sulfated beef (SB) insulins in patients with insulin-antibody–mediated insulin resistance.

RESEARCH DESIGN AND METHODS — A randomized double-blind sequential crossover study was performed with each insulin administered for 56 days unless dose reached 200 U/day or allergy developed. Participants were 26 individuals with history of B-P insulin dosage ≥200 U/day and insulin binding capacities >0.216 nM (30 mU/ml serum). Twenty-one participants completed the study. Insulin dosage/day, fasting plasma glucose, percentage HbA_1, insulin antibody binding capacity (IABC), bound insulin (BI), percentage binding of ^{125}I-labeled B, P, and H insulins, and receptor inhibition factor (RIF) were assessed.

RESULTS — Mean insulin dosage (U/day) was significantly greater on B (88.9) than on P (29.2), H (29.4), or SB (29.6). On B, dosage increased in 12 individuals and reached 200 U/day in 6 individuals. Mean fasting plasma glucose (12.1 mM) and HbA_1 (11%) were significantly higher on B than on P, H, and SB. Mean IABC, bound insulin, RIF, and percentage of B, P, and H bound were significantly higher on B than on P, H, and SB. Prolonged treatment with SB before entry into the study (>5 wk) resulted in a blunted anamnestic response to B insulin.

CONCLUSIONS — Rechallenge with B results in anamnestic immunological response and deterioration of metabolic control. SB, H, and P insulins have equivalent effects in patients with insulin antibody–mediated immunologic resistance.

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Insulin antibodies often have an adverse effect on metabolic control (1). Antibody-induced resistance has been treated with steroids, concentrated (500 or 5000 U) beef (B) or pork (P) insulin, desalanated P insulin, sulfated beef (SB) or sulfated P insulin, fish insulin, pancreatic human (H) insulin, immunosuppressants, and human insulin (rDNA) (2,3). The objective of this double-blind cross over trial was to measure the effects on immunogenicity and metabolic control of purified regular B, P, H (rDNA), and SB insulin therapies in a group of individuals with a history of immunologic insulin resistance.

RESEARCH DESIGN AND METHODS

— Participants were 26 individuals treated with SB for immunological insulin resistance (a daily insulin dose of ≥200 U/day plus an insulin antibody binding capacity (IABC) >0.216 nM (30 mU/ml serum); 8 for >1 yr (54–708 wk), 11 for 1–5 wk, and 2 for <1 wk (2–4 days). Twenty-one participants completed the study. Mean entry age was 60 yr (range 34–75 yr), duration of diabetes was 13.7 yr (range 2–30 yr), and initial body mass index (kg/m^2) was 25.0 (range 17.5–34.0 kg/m^2). Neutral regular B and P (both containing <3 ppm proinsulin), H (Lilly, Indianapolis, IN), and SB insulin (IND 17805; Connaught, Willowdale, Ontario, Canada) were placed in coded vials. Patients were randomly assigned to one of eight treatment sequences consisting of 56 days on each insulin and administered at 0700, 1500, and 2300 daily. Dosages were adjusted to attain fasting and premeal plasma glucose between 4 and 10 mM. If the dose requirement reached 200 U/day or if allergy developed, the patient was moved to the next insulin. Fasting venous plasma glucose levels and sera for insulin antibody measurements were collected at baseline and then every 2 wk. Total HbA_1 (normal range 4.5–8.5%) was measured at baseline and after each period. IABC in unextracted sera was measured by a previously published method

Table 1—*Insulin dose and metabolic control*

	INSULIN (U/DAY)	FASTING PLASMA GLUCOSE (MM)	HbA_1 (%)
BASELINE		10.9 ± 3.7	11.0 ± 2.2
BEEF (B)	88.9 ± 79.3	12.1 ± 5.5	11.0 ± 2.4
PORK (P)	29.2 ± 16.8	8.9 ± 2.9	8.9 ± 1.4
HUMAN (H)	29.4 ± 16.9	10.4 ± 3.6	9.1 ± 1.7
SULFATED B	29.6 ± 17.9	9.0 ± 3.4	9.5 ± 1.5
P	<0.001 (B > other)	0.003 (B > P, SB)	<0.001 Baseline, B > P,H,SB

Values are means ± SD.

(2,4). Antibody bound insulin (BI) in serum was determined with a polyethylene glycol method (5). Percentage binding of serum antibodies to ^{125}I-labeled B, ^{125}I-labeled P, and ^{125}I-labeled H insulin was measured in acid charcoal-extracted sera (5). The inhibitory effects of patient sera on ^{125}I-labeled insulin binding by insulin receptors of IM-9 lymphocytes was measured (6) and was termed *receptor inhibition factor* (RIF; the reciprocal dilution of the patient's serum resulting in 50% inhibition of ^{125}I-insulin binding to IM-9 cells).

Statistical analysis

Data are means ± SD. IABC, BI, RIF, and percentage bound underwent log transformation to normalize distributions. Repeated-measures analysis of variance and the Newman-Keul's procedure at $P = 0.05$ was used to analyze these variables (7). Relationships between variables were examined with Pearson's and Spearman's rank correlation coefficients. Changes during follow-up or due to B insulin therapy were analyzed with a paired *t* test.

RESULTS— At the end of the treatment period, the mean dose of B was significantly greater than the mean dose on P, H, and SB. Fasting plasma glucose was significantly higher on B than on P and SB, and HbA_1 was significantly higher on B than on P and H (Table 1). Table 2 shows that IABC, BI, RIF, and percentage H bound were greater while on B than on all other therapies. While on B, patients fell into three groups: in nine patients, no dose increase or change in control; in five, a dose increase of <100 U/day; and in seven, a dose increase of >100 U/day and an increase in mean glucose and HbA_1. The dose reached 200 U/day in 6 of the latter (Table 3). Prestudy SB therapy for >1 yr prevented recurrence of immunological insulin resistance on subsequent B, but SB therapy for ≤5 wk did not. One female patient developed generalized urticaria on SB and then immunologic resistance on B. One male patient developed immunologic resistance (200 U/day) on B, and was hospitalized with diabetic ketoacidosis. After completion, patients were transferred to NPH and/or regular H. One patient died 8 mo later. The other 20 patients at 1-yr follow-up had decreased mean IABC from 1466 to 590 nM ($P < 0.001$).

During 2 mo on P, H, and SB, no individual developed recrudescent immunological resistance. While on B, 12 of 21 patients required an increase in dose and 6 developed recurrent immunological insulin resistance (≥200 U/day). An increase in fasting plasma glucose and HbA_1 was associated with a marked dose increase (Table 3). Recurrent immunological resistance on B therapy occurred in as little as 18 days. During 1 yr of follow-up therapy on H, the IABC decreased significantly, as has been observed by others (8). We noted that SB therapy for >1 yr but not for <6 wk reduced the risk of recurrent immunological resistance on subsequent B therapy. SB therapy for 1 yr has been associated with a virtual disappearance of T-cell and antibody responses to B, P, and H in parallel with the appearance of insulin-specific $CD8^+$ suppressor T cells (9). In five individuals with persistent immunological resistance, transfer to SB resulted in a remission of immunological responses to B, P, and H (10).

CONCLUSIONS— This study has shown an anamnestic antibody response in individuals rechallenged with B. Prolonged treatment with SB may depress the potential for immunological resistance upon rechallenge with B, and pro-

Table 2—*Insulin antibody measurements*

	IABC*	BI*	HUMAN INSULIN (%)†	RIF*
BASELINE	3.141 ± 0.458 (1382)	0.141 ± 0.458 (1.38)	57.0 ± 26.7	2.20 ± 0.67 (158)
BEEF	3.292 ± 0.326 (1957)	0.292 ± 0.326 (1.96)	74.9 ± 10.9	2.69 ± 0.47 (490)
PORK	3.103 ± 0.498 (1269)	0.103 ± 0.489 (1.27)	54.9 ± 20.9	2.01 ± 0.53 (102)
HUMAN	3.077 ± 0.517 (1194)	0.077 ± 0.517 (1.19)	57.8 ± 19.2	2.34 ± 0.58 (219)
SULFATED BEEF	3.076 ± 0.496 (1190)	0.076 ± 0.496 (1.19)	51.9 ± 19.6	2.24 ± 0.72 (174)
P	0.005 B > others	0.005 B > others	<0.001 B > others	<0.001 B > others

Values are means ± SD with geometric means in parentheses. IABC, insulin antibody binding capacity; BI, bound insulin; RIF, receptor inhibition factor.
*A log transformation was conducted to normalize data.
†Percentage of SB and P insulins bound were not distinguishable from percentage human insulin bound.

Table 3—*Changes from pre- to postbeef insulin therapy*

PATIENTS (N)	INSULIN DOSE (U/DAY)	FASTING PLASMA GLUCOSE (MM)	HbA_1 (%)
9	0	0	−0.5 ± 1.8
5	+42 ± 30*	−4.6 ± 2.0*	−1.4 ± 0.7†
7	+141 ± 25*	+6.1 ± 3.2*	+1.5 ± 1.6†

Data are given means ± SD. Significance levels are for a change from pre- to postbeef insulin therapy.
*$P < 0.001$.
†$P < 0.01$.

longed treatment with H resulted in substantial reduction of IABC. Rechallenge of insulin-antibody–resistant individuals with B is ill advised. Treatment of patients with immunological resistance with P or H is equally effective but SB remains an option in patients with persistent resistance to B, P, or H.

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