Concentration of the Complement Activation Product, Acylation-Stimulating Protein, Is Related to C-Reactive Protein in Patients With Type 2 Diabetes

Pertti Ebeling, Anna-Maija Teppo, Heikki A. Koistinen, and Veikko A. Koivisto

Type 2 diabetes is characterized by increased acute phase serum proteins. We wanted to study how these proteins are related to complement activation in type 2 diabetes and how improvement of glycemic control affects them or complement activation. A total of 29 type 2 diabetic patients (age, 55.2 ± 1.8 years, glycosylated hemoglobin [HbA_{1c}] 8.9% ± 0.2%, body mass index [BMI] 30.9 ± 0.8 kg/m^2, duration 5.9 ± 1.3 years) participated in the study. They were previously treated either with diet alone or in combination with 1 oral antihyperglycemic medication. After a period of at least 4 weeks run-in on diet only, the patients were randomized to pioglitazone, glibenclamide, or placebo. Blood samples were taken before the treatments and at the end of the 6-month therapy. Basal C-reactive protein (CRP) level was related to acylation-stimulating protein (ASP) concentration ($r = .55$, $P < .01$), and many acute phase serum protein concentrations were associated with each other. The treatment reduced HbA_{1c} level in the pioglitazone (from 9.1 ± 0.3% to 8.0 ± 0.5%, $P < .05$) and glibenclamide (from 8.9% ± 0.3% to 7.7% ± 0.2%, $P < .05$) groups. Glibenclamide treatment was associated with a reduction in α-1-antitrypsin ($P < .05$), ceruloplasmin ($P < .01$), and complement C3 protein (C3) ($P < .05$). Although ASP did not change significantly in any of the treatment subgroups, in the whole patient population, the change in HbA_{1c} during the treatments correlated positively with the change in ASP, ($r = .43$, $P < .05$). The changes in many acute phase serum proteins and ASP were related to each other. In conclusion, (1) inflammatory factors and complement activation are associated in patients with type 2 diabetes, and (2) changes in hyperglycemia are related to changes in the concentration of the complement activation product, ASP.

THE ROLE OF INFLAMMATORY factors in many diseases, including type 2 diabetes, has become the focus of great interest during the last few years. Increased levels of haptoglobin, α-1-acid glycoprotein (A1GP), C-reactive protein (CRP), serum amyloid A (SAA), and interleukin-6 (IL-6) have been reported in type 2 diabetes or in impaired glucose tolerance.[1,2] Also atherosclerosis, which is often associated with type 2 diabetes, is increasingly considered to be a chronic inflammatory disease.[3] Likewise, complement activation is associated with atherosclerosis.[4] Insulin resistance is associated both with type 2 diabetes and with cardiovascular disease. The mechanisms of how inflammation is associated with these diseases are still largely unknown, but a postulation of how inflammation is related to type 2 diabetes[5] has been presented. The inflammatory cytokine, tumor necrosis factor-α (TNF-α),[6] and complement C3 protein (C3) concentrations[7] are closely related to insulin resistance and obesity. CRP functions in close collaboration with complement already in early atherosclerosis.[8] Acylation-stimulating protein (ASP) is the cleavage product of C3.[9] It is formed from the anaphylatoxin C3a and has a pivotal function in triglyceride synthesis within adipocytes.[9] It also exerts immunomodulatory action.[10]

Many antihyperglycemic medications also have extrapancreatic effects. Thiazolidinediones are a new group of pharmaceutical agents, with both glucose and lipid-lowering effects and the ability to reduce insulin resistance.[11] Recent in vitro data suggest that thiazolidinediones inhibit the production of inflammatory cytokines, as well as downstream markers of inflammation, such as nitric oxide produced by monocytic cells.[12,13] Data in the Wistar fatty rats show that the antidiabetic and lipid-lowering action of a thiazolidinedione (pioglitazone) is associated with the suppression of TNF-α production.[14] Although the main antihyperglycemic effects of sulfonylureas are usually attributed to increased insulin production, they may also possess extrapancreatic effects.[15-18] We have recently shown that troglitazone, in addition to improving glycemic control, selectively reduces acute phase serum proteins in patients with type 2 diabetes.[19]

We hypothesized that inflammation (as assessed by acute phase proteins) and consequent activation of the complement (as assessed by ASP) characterize type 2 diabetes and that normalization of these parameters would occur with treatment, which effected improved glycemic control. Consequently, in the present study, we wanted to study 3 things. First, are inflammatory factors and activation of the complement related? Second, how does improvement of glycemic control by pioglitazone or glibenclamide affect concentrations of acute phase serum proteins (SAA, CRP, A1GP, α-1-antitrypsin, C3, ceruloplasmin, haptoglobin)? Because many acute phase proteins are regulated differently, we chose many markers of inflammation to get a comprehensive picture of the possible antiinflammatory effects. Third, is improved metabolic control related to changes in complement activation?

PATIENTS AND METHODS

Patients

A total of 29 patients (21 males, 8 females) with type 2 diabetes participated in this study. The patient characteristics are shown in Table 1. This study consisted of patients from Helsinki University Hospital, who took part in a phase III study comparing the effects of pioglitazone, glibenclamide, and placebo on glucose control. The inclusion criteria for the main study were: type 2 diabetes treated with diet and 1 oral medication or diet alone; body mass index (BMI), ≥ 25 kg/m^2;

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Table 1. Characteristics of the Patients Before the Treatments

Age (yr)	55.2 ± 1.8
BMI (kg/m^2)	30.9 ± 0.8
Diabetes duration (yr)	5.9 ± 1.3
Fasting serum glucose (mmol/L)	11.2 ± 0.3
HbA_{1c} (%)	8.9 ± 0.2

age, 35 years or older and 75 years or younger; glycosylated hemoglobin (HbA_{1c}), ≥ 7.5%; and fasting serum glucose, ≥7.8 mmol/L. Many patients used concomitant medication, mainly antihypertensives, during the study. This medication was the same before and at the end of the study.

Design

This study was performed in a randomized double-blind manner. At the beginning of the study, a fasting blood sample for serum glucose and HbA_{1c} determination was taken for the central laboratory. One week later, blood samples were taken for the measurements made at the research laboratory of Helsinki University Hospital, and the patients were randomized to pioglitatzone (30 mg), glibenclamide (2.5 mg), or placebo. The medication was taken once daily. The patients visited the outpatient clinics at 2- to 6-week intervals for safety measurements. If the reduction of HbA_{1c} at week 9 was not greater than or equal to 0.3%, the antidiabetic medication was doubled in the glibenclamide group (to 5 mg) and increased to 45 mg in the pioglitatzone group. The dose was increased in 4 of 9 patients on pioglitazone, in 7 of 10 patients on placebo, and in none of the patients on glibenclamide. After 6 months of therapy, blood samples were taken for glucose and HbA_{1c} measurements in the central laboratory and for other measurements, which were made at the research laboratory of Helsinki University Central Hospital. At all visits, signs and symptoms of infections, inflammation, and other adverse events were evaluated. The study was approved by the Ethical Committee of Helsinki University Hospital. Each patient gave his/her written, informed consent, and the study was conducted according to the rules of Good Clinical Practice.

Methods

HbA_{1c} (reference range, 4.3% to 6.1%),[20] serum insulin, C-peptide, total and high-density lipoprotein (HDL)-cholesterol, triglycerides, and free fatty acid (FFA) concentrations were determined as previously described.[21] Plasma ASP was determined with enzyme-linked immunosorbent assay (ELISA) (Quidel, San Diego, CA) from samples immediately stored after sampling and kept in −70°C until analyzed. The manufacturer reported mean ASP concentration in 20 healthy blood donors was 77 μg/L (range, 26 to 146). Serum haptoglobin (reference range, 0.29 to 2.00 g/L), A1GP (reference range, 500 to 1,200 mg/L), α-1-antitrypsin (reference range, 0.98 to 1.78 g/L), ceruloplasmin (reference range, 200 to 550 mg/L), and C3 (reference range, 0.7 to 1.6 g/L) concentrations were determined with automated immunoturbidometric methods (Hitachi 911, Tokyo, Japan). SAA was measured by enzyme immunoassay (Cytoscreen; Biosource International, Camarillo, CA), CRP by radioimmunoassay as previously described.[22] The detection limit for SAA was 0.005 mg/L and for CRP 0.05 mg/L. In 50 healthy subjects (33 males, 17 females; age, 38 ± 2 years), the mean ± SEM for SAA was 2.1 ± 0.5 mg/L and for CRP 2.7 ± 0.5 mg/L.

Statistical Analysis

The statistical differences between concentrations before treatment and at the end of treatment were calculated using Wilcoxon's signed test. The correlation analysis was performed with Spearman's test. The proportion of variation in the dependant variables explained by the variations in the independent variables was calculated by forward stepwise multiple linear regression analysis using adjusted squared multiple *R*. *P* values less than .05 were considered statistically significant. The results are given as mean ± SEM.

RESULTS

At randomization, the C3 concentration (1.55 ± 0.05 g/L) was near the upper limit of our reference range and the mean SAA concentration (13.5 ± 2.7 mg/L [median, 8.0 mg/L]) was higher than in our younger reference group. Before the treatments, BMI was related to C3 ($r = .76$, $P < .001$) and haptoglobin ($r = .47$, $P < .05$). At randomization, CRP was related to ASP (Fig 1) and several other acute phase serum proteins (Table 2). Many acute phase serum proteins were related to each other (Table 2).

In the whole group, the treatments resulted in improved glucose control as indicated by reduced HbA_{1c} (8.0% ± 0.2%, $P < .01$ *v* baseline) and glucose (9.8 ± 0.3 mmol/L, $P < .001$ *v* baseline). The effects of treatments on different parameters in the patient groups are given in Table 3. In the placebo group, the reduction in fasting glucose did not quite reach statistical significance ($P = .07$). Glibenclamide treatment was also associated with a reduction in C3. Of the measured acute phase proteins A1GP ($P < .05$), C3 ($P < .05$), haptoglobin ($P < .05$), and ceruloplasmin ($P < .01$) were reduced when the patient populations were combined.

The change in HbA_{1c} during the treatments was significantly related to the change in ASP (Fig 2) and α-1-antitrypsin ($r = .47$, $P < .02$), but not to changes in A1GP ($r = .35$), ceruloplasmin ($r = .31$), CRP ($r = .34$), haptoglobin ($r = .31$), SAA ($r = .32$), or C3 ($r = .08$). The changes in many acute phase serum proteins and ASP were related to each other (Table 2). In regression analysis, the change in HBA_{1c} was best explained by changes in ASP and insulin (negative), which together explained 33% of the variation of the change in HbA_{1c} ($P < .01$). When the factors related to the change in ASP were calculated with regression analysis, the changes in α-1-antitrypsin and HbA_{1c} accounted for 40% of the variation of the change in ASP. ASP and CRP also correlated significantly after treatments ($r = .56$, $P < .01$).

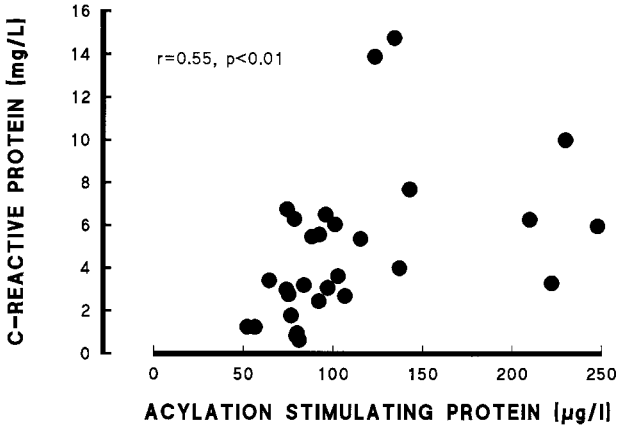

Fig 1. Correlation between concentrations of CRP and ASP before the treatments.

Table 2. Correlation Coefficients Between Concentrations of Different Serum Proteins Before the Treatments (lower left half) and Between Changes of Different Serum Proteins During the Treatments (upper right half)

	A1GP	Antitrypsin	ASP	Cerulopl	C3	CRP	Haptoglobin	SAA	
A1GP		0.626*	0.470†	0.744*	0.204	0.523‡	0.675*	0.438†	
Antitrypsin	0.048		0.600*	0.708*	0.456‡	0.534[2]	0.613*	0.545‡	Change
ASP	0.353	0.507‡		0.478†	0.178	0.303	0.327	0.308	During
Cerulopl	0.335	0.410†	0.653*		0.284	0.552‡	0.657*	0.407†	Treatment
C3	0.489‡	−0.103	0.139	0.400†		0.046	0.384	0.482†	
CRP	0.435†	0.455†	0.553‡	0.813*	0.348		0.496‡	0.643*	
Haptoglobin	0.623*	0.177	0.225	0.352	0.554‡	0.544‡		0.328	
SAA	0.374†	0.400†	0.465†	0.537‡	0.341	0.589*	0.376†		
				Baseline					

Abbreviations: A1GP, α-1-acid glycoprotein; antitrypsin, α-1-antitrypsin; ASP, acylation-stimulating protein; cerulopl, ceruloplasmin; C3, complement protein C3; CRP, C-reactive protein; SAA, serum amyloid A.

* $P < .001$.

† $P < .05$.

‡ $P < .01$.

In the pioglitazone group, the reduction in HbA_{1c} was related to the reduction in SAA ($r = .82$, $P < .05$), but the correlation to the reduction in haptoglobin ($r = .75$), antitrypsin ($r = .72$), A1GP ($r = .64$), and ceruloplasmin ($r = .64$) did not correlate significantly. In the glibenclamide group, there was no significant relationship between reduction in HbA_{1c} and reduction in antitrypsin ($r = .49$) or other acute phase proteins ($r < .3$ for all). In the placebo-treated group, the correlation coefficient between change in HbA_{1c} and inflammatory parameters was less than .4 for all.

DISCUSSION

In the current study, we investigated if inflammatory factors and activation of the complement are related in patients with type 2 diabetes. We also examined how improvement of glycemic control affects serum concentrations of acute phase proteins and complement activation. Three treatments were used to achieve better glycemic control. Of these, pioglitazone and glibenclamide achieved this goal, while placebo treatment had no effect on glycemic control. The effects of these different

Table 3. Effect of the Treatments on Various Measured Parameters

	Pioglitazone (n = 9)		Glibenclamide (n = 10)		Placebo (n = 10)	
	Before Treatment	Treatment	Before Treatment	Treatment	Before Treatment	Treatment
HbA_{1c} (%)	9.1 ± 0.3	8.0 ± 0.5‡ (n = 8)	8.9 ± 0.3	7.7 ± 0.2‡	8.6 ± 0.2	8.4 ± 0.3
f-glucose (mmol/L)	10.9 ± 0.6	9.5 ± 0.8§	11.6 ± 0.5	10.1 ± 0.6	11.3 ± 0.5	9.9 ± 0.3
Insulin (pmol/L)	58.7 ± 9.1	50.7 ± 5.1	88.2 ± 19.9	90.0 ± 16.1	78.6 ± 11.5	76.2 ± 17.2
C-peptide (nmol/L)	0.88 ± 0.10	0.79 ± 0.06	1.05 ± 0.14	1.06 ± 0.12	1.10 ± 0.13	1.01 ± 0.11
BMI (kg/m^2)	30.5 ± 1.3	31.4 ± 1.5‡	30.2 ± 1.7	31.0 ± 1.6§	31.9 ± 1.5	31.3 ± 1.3
A1GP (mg/L)*	849 ± 78	814 ± 91	838 ± 57	761 ± 46	852 ± 65	798 ± 78
α-1-antitrypsin (g/L)*	1.06 ± 0.07	1.12 ± 0.07	1.09 ± 0.05	1.02 ± 0.03‡	1.12 ± 0.07	1.02 ± 0.06
C3 (g/L)	1.53 ± 0.09	1.53 ± 0.07	1.62 ± 0.10	1.47 ± 0.09‡	1.56 ± 0.07	1.49 ± 0.04
ASP (ng/mL)	121 ± 23	109 ± 13	115 ± 18	103 ± 17	98 ± 9	114 ± 10
Ceruloplasmin (mg/L)*	318 ± 22	318 ± 24	320 ± 11	284 ± 13§	348 ± 23	297 ± 20
CRP (mg/L)*†	3.07	3.34	5.81	2.82	4.69	2.36
	(1.25-14.76)	(1.64-9.50)	(0.64-10.00)	(0.36-11.7)	(0.95-13.89)	(0.98-7.34)
Haptoglobin (g/L)*	1.12 ± 0.18	1.21 ± 0.22	1.23 ± 0.20	1.03 ± 0.16	1.24 ± 0.15	1.12 ± 0.18
SAA (mg/L)*†	6.50	10.65	10.10	6.90	7.75	8.20
	(2.0-55.0)	(1.6-33.0)	(2.0-22.0)	(1.6-50.0)	(1.5-60.0)	(2.6-17.0)
Cholesterol (mmol/L)	5.18 ± 0.22	5.83 ± 0.21‡	5.37 ± 0.18	5.74 ± 0.27‡	5.20 ± 0.30	5.82 ± 0.34‡
HDL-C (mmol/L)	1.19 ± 0.07	1.41 ± 0.08‡	1.17 ± 0.06	1.15 ± 0.07	1.22 ± 0.08	1.25 ± 0.09
Triglycerides (mmol/L)	2.34 ± 0.47	1.97 ± 0.29	2.05 ± 0.35	2.29 ± 0.52	2.19 ± 0.35	2.48 ± 0.34
FFA (μmol/L)	672 ± 96	770 ± 130	825 ± 89	725 ± 60	940 ± 107	719 ± 72

Abbreviations: A1GP, α-1-acid glycoprotein; ASP, acylation-stimulating protein; C3, complement C3 protein; CRP, C-reactive protein; FFA, free fatty acids; HDL-C, high-density lipoprotein-cholesterol; SAA, serum amyloid A.

* Two treated patients (1 pioglitazone, 1 placebo) had mild clinical inflammation and their acute phase proteins not included in the treated groups.

† Skewed data, median, and range given.

‡ $P < .05$.

§ $P < .01$.

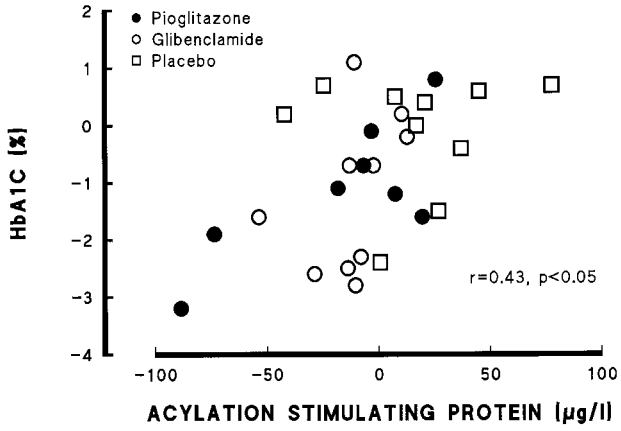

Fig 2. Correlation between changes in HbA_{1c} and ASP concentration during the treatments.

treatments on concentrations of acute phase proteins were also studied. It is important to know that, because the drugs have different mechanisms of action, their possible effects on inflammatory parameters are also probably different.

The correlation between CRP and ASP (Fig 1) before the treatments is a very interesting finding. While CRP is a classical marker of inflammation,[23] ASP is the cleavage product of C3,[9] and it has a pivotal function in triglyceride synthesis within adipocytes.[9] Elevated ASP (or C3adesArg) has been considered as a sign of accelerated activation of the complement.[24] It is particularly important that this correlation is found in people without any clinical inflammation or infection. Previously an association between these 2 systems has been shown in patients with sepsis.[25] Furthermore, it is known that CRP is able to activate complement[23,25] providing a possible mechanism for this association.

The treatments resulted in improved glucose control in the whole patient population, but this improvement mainly accounted for the changes in the pioglitazone and glibenclamide groups (Table 3). This change in the combined group was accompanied by a reduction in A1GP, C3, haptoglobin, and ceruloplasmin. All of these and many other acute phase proteins are very stable in patients with type 2 diabetes for at least 5 months.[19] When the effects of different treatments on acute phase serum proteins were studied, differences in the effects of treatments became visible. Glibenclamide treatment was associated with statistically significant reductions in C3, α-1-antitrypsin and ceruloplasmin, while no significant changes in acute phase proteins were seen in the other treatment groups (Table 3). The reduction of C3 in our glibenclamide-treated patients was seen in the face of increased BMI during the treatment. Higher BMI is associated with higher C3 concentration,[19,26] as was also shown in this study.

The degree of improvement in metabolic control was related to the reduction in complement activation, as shown by the association between changes in HBA_{1c} and ASP (Fig 2). The change in HbA_{1c} was also associated with the change in α-1-antitrypsin. The relationship between changes in HBA_{1c} and ASP may depend on several different things. Reduction of some underlying factors, such as inflammation, could simultaneously reduce both of these. In our previous study, troglitazone reduced C3, SAA, and AIGP in insulin-treated patients with type 2 diabetes in conjunction with reduced glycemia.[19] Of these 3 acute phase serum proteins, C3 and A1GP were reduced in the combined group in the present study. Also, changes in many acute phase serum proteins and ASP were related to each other. However, the correlation between changes in inflammatory factors and glycemia were not stronger than changes in HBA_{1c} and ASP, suggesting other mechanisms of association. In the regression analysis, the variation in ASP was best explained by variations in α-1-antitrypsin and HbA_{1c}. This could be interpreted that changes in the degree of inflammation contribute to the activation of the complement. The contribution of variation in HBA_{1c} to variation in ASP is a very intriguing finding in light of the recent information on the complement system. In addition to the well-known classical and alternative pathways, the complement system can also be activated via the mannose-binding lectin (MBL) pathway.[27,28] MBL has specific binding to mannose and N-acetylglucosamine,[29] a factor associated with insulin resistance.[30] The correlation between degree of improvement in glycemic control and complement activation supports a possible role of hyperglycemia as an accelerator of complement activation, in agreement with a recent in vitro study.[31] As the association in improvement of glycemic control with changes in acute phase proteins was calculated, only in the pioglitazone-treated group was a significant correlation found. The reduction in HbA_{1c} was related to the reduction in SAA. In the patients with the most reduced SAA, HBA_{1c} also improved the most. Due to the small number of patients in the treatment groups, only correlations with a high correlation coefficient were significant.

When considering possible mechanisms of antihyperglycemic action, 2 things are important. First, the different treatments probably have different mechanisms of action. Second, the small number of patients, especially in the individual treatment groups, enables only the perception of major changes in the measured parameters. It has recently been shown that preceding high A1GP concentration is a risk factor for type 2 diabetes.[32] This is in accordance with the reported increased levels of haptoglobin, A1GP, CRP, SAA, and IL-6 in type 2 diabetes or in impaired glucose tolerance.[1,2] Therefore, reduction of inflammation might be 1 plausible mechanism. Thiazolidinediones have antiinflammatory effect both in vitro[12,13] and in vivo.[19] In this study, we did not see a reduction in acute phase serum proteins in the pioglitazone-treated group (Table 3), although SAA was reduced in 6 of 8 patients. The effects of different thiazolidinediones are not identical. However, when the changes in glycemia and SAA were compared, the patients with the greatest reduction in SAA also tended to have greatest reductions in HbA_{1c}. Therefore, a larger study is needed to settle this question. Regarding mechanisms responsible for the antihyperglycemic action of glibenclamide, Table 3 shows that fasting insulin was not changed. This is in accordance with previous studies showing only small effects on fasting serum insulin levels.[18] The unchanged fasting insulin level is also in agreement with the extrapancreatic effects of glibenclamide as reported in healthy rats[16] and in patients with type 2 diabetes.[17,18]

Taken together, our study suggests that inflammatory factors

are associated with complement activation in patients with type 2 diabetes with no signs of clinical inflammation or infection. Changes in glycemia were related to changes in complement activation, revealing a previously unreported association between glucose metabolism and the complement system. The reduction in some, but not all, acute phase serum proteins in the glibenclamide group is in accordance with previous reports also showing extrapancreatic effects for glibenclamide.

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