MPO Activity and Generation of Active O_2 Species in Leukocytes From Poorly Controlled Diabetic Patients

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OBJECTIVE — This study was undertaken to determine which part of ROI generation is reduced in the neutrophils from patients with NIDDM.

RESEARCH DESIGN AND METHODS — Superoxide anion (O_2^-) production, LDCL activity in response to opsonized zymosan, and MPO activity were measured in leukocytes of poorly controlled NIDDM patients (FBG >8.89 mM, HbA_1 >10%).

RESULTS — In diabetic subjects, both O_2^- production and LDCL activity assessed with initial slope gradient and peak value were significantly reduced. MPO activity was also decreased in diabetic subjects, and there was a significant correlation between HbA_1 levels and MPO activity of diabetic subjects.

CONCLUSIONS — This study demonstrated that every step in leukocyte ROI generation should be reduced in the leukocytes from poorly controlled diabetic patients.

Poorly controlled diabetic patients are prone to infection, which may be an important risk factor of increased mortality (1,2). The peak value of LDCL has been reported to be reduced in leukocytes of patients with diabetes mellitus (3,4). Hyperglycemia after streptozocin administration interfered with leukocyte phagocytic function assessed with LDCL activities, and insulin supplementation increased LDCL activities of leukocytes in a dose-dependent manner (5). Because glucose transport of polymorphonuclear leukocytes was increased in patients with NIDDM (6), hyperglycemia may affect phagocytic function in leukocytes from NIDDM patients. This study was designed to investigate which part of ROI generation is reduced in leukocytes from poorly controlled NIDDM patients.

RESEARCH DESIGN AND METHODS

— The blood samples were collected from 18 poorly controlled NIDDM patients (8 men, 10 women; 43.8 ± 2.3 yr old, HbA_1 >10%, and FBG >8.89 mM) and 20 normoglycemic healthy nondiabetic volunteers (11 men, 9 women; 43.2 ± 2.8 yr old). The average duration after diagnosis of diabetes mellitus was 8.4 ± 2.3 yr. The average FBG of diabetic patients was 10.89 ± 1.58 mM, and HbA_1 was 12.5 ± 1.7%. FBG and HbA_1 of healthy nondiabetic control subjects were 4.79 ± 0.47 mM and 5.6 ± 0.3%, respectively. Within 1 mo before the day of blood collection, none of the patients had suffered from any infectious diseases. Neither serum C-reactive protein nor urine ketone bodies were detected. Whole blood was collected into heparinized syringe under fasting conditions. The leukocytes were prepared with Dextran 70 (6% Dextran [wt/vol] in 0.9% NaCl) (7). The cells obtained consisted of 85–90% polymorphonuclear leukocytes.

The O_2^- production in the prepared leukocytes was measured by the reduction of exogenously added ferricytochrome c (5,7). Briefly, the standard reaction mixture, which consisted of 10^7 leukocytes and 50 μM ferricytochrome c, was agitated in an incubator at 37°C. The inducer consisted of 4 mg opsonized zymosan, 4 μg phorbol, or 80 μmol NaF with or without inhibitor in 4 ml Krebs-Ringer phosphate buffer (pH 7.4) containing 0.2% glucose and 0.2% bovine serum albumin. The inducer was added at zero time, and a 1.2-ml aliquot was taken immediately (time 0). After incu-

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NIDDM, NON-INSULIN-DEPENDENT DIABETES MELLITUS; LDCL, LUMINOL-DEPENDENT CHEMILUMINESCENCE; FBG, FASTING BLOOD GLUCOSE; MPO, MYELOPEROXIDASE; ROI, REACTIVE OXYGEN INTERMEDIATE.

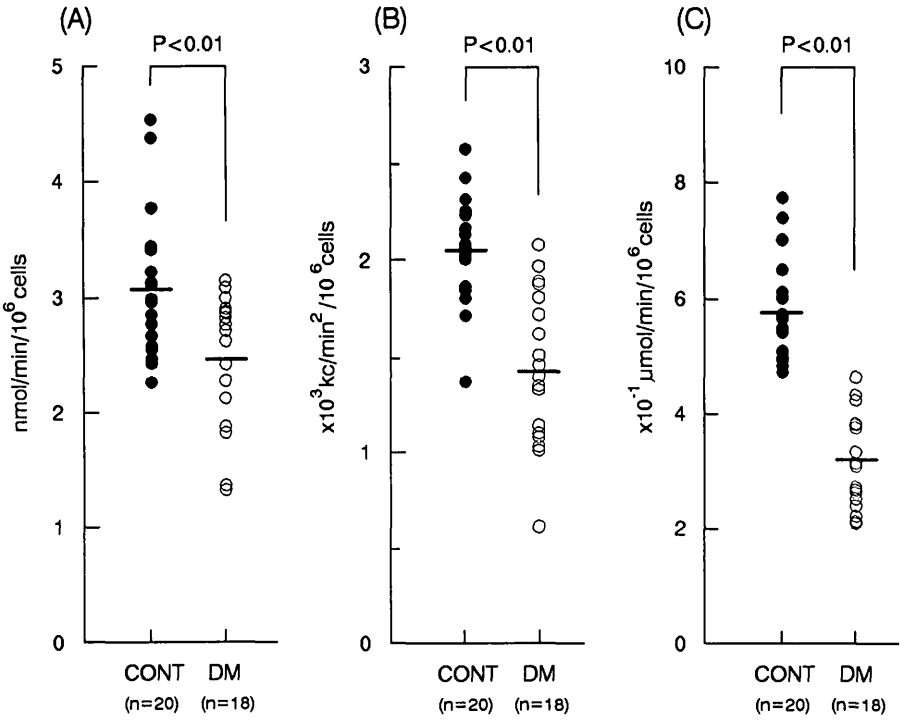

Figure 1—*Production of O_2^- (A), LDCL activity (B), and MPO activity (C) in leukocytes from control subjects and diabetic patients (DM).*

bation, the tubes were centrifuged at 700 × *g* for 10 min in the cold room. Absorbances of the supernatant solutions were determined spectrophotometrically at 550 nm.

The method for the measurement of LDCL activity in the leukocytes was described previously (5,7). One hundred microliters or 500 μl leukocyte-suspended solutions were transferred into the sample vial tube, and 100 μl of 50 μM luminol was added. After preincubation at 37°C, the reaction was started by the addition of 4 mg zymosan (dissolved into 100 μl Hanks' buffer). For the measurement of MPO activity, the LDCL activity was assessed with the initial slope gradient and peak values. The 0.2-mM phosphate buffer, 40 mM guayacol, and 0.02% Setaburon solution were added to the 1 × 10^4-cell–disrupted solution. After addition of 0.5 mM H_2O_2, changes of absorbances after the production of tetraguayacol were measured with a spectrophotometer.

All data are means ± SE. Statistical analysis was performed by analysis of variance followed by unpaired *t* test for individual comparisons of the means.

RESULTS— The production of O_2^- was reduced in leukocytes from the diabetic group (2.49 ± 0.14 nmol · min^{-1} · 10^{-6} cells) compared with nondiabetic control subjects (3.07 ± 0.14 nmol · min^{-1} · 10^{-6} cells) (Fig. 1*A*). In addition, the LDCL activity assessed with initial slope gradient was significantly ($P < 0.01$) lower in the diabetic group (1.43 ± 0.10 kc · min^{-2} · 10^{-6} cells) compared with nondiabetic volunteers (2.06 ± 0.06 kc · min^{-2} · 10^{-6} cells) (Fig. 1*B*). In addition, the peak values of diabetic patients (2.84 ± 0.48 kc · min^{-1} · 10^{-6} cells) were lower than those of control subjects (3.90 ± 0.40 kc · min^{-1} · 10^{-6} cells).

A marked reduction of MPO activity was observed in leukocytes from diabetic patients (3.15 ± 0.19 × 10^{-1} μmol · min^{-1} · 10^{-6} cells) compared with control subjects (5.72 ± 0.21 × 10^{-1} μmol · min^{-1} · 10^{-6} cells) (Fig. 1*C*). Although the K_m of MPO in diabetic patients (1.83 ± 0.03 × 10^{-1} μM) was not different from that in control subjects (1.78 ± 0.04 × 10^{-1} μM), V_{max} of diabetic patients (0.61 ± 0.97 μmol · min^{-1} · 10^{-6} cells) was significantly lower than that of control subjects (0.93 ± 0.11 μmol · min^{-1} · 10^{-6} cells). There was a significantly negative correlation between MPO activity and HbA_1 level in diabetic patients ($r = -0.874$, $P < 0.01$; Fig. 2) and in a whole group of this study ($r = -0.923$, $P < 0.01$).

CONCLUSIONS— This study showed that O_2^- accumulation, LDCL activity, and MPO activity were significantly reduced in poorly controlled diabetic patients. The reduction of LDCL activity assessed with initial slope gradient and peak value in diabetic subjects was consistent with the previous observations in diabetic subjects (3,4) and experimental diabetic animals (5). This study added a finding that both O_2^- accumulation and MPO activity were also decreased in the leukocytes from diabetic patients.

It has been reported that both O_2^- generation and MPO-H_2O_2-Cl^- systems are involved in oxidation-dependent microbicidal mechanisms of

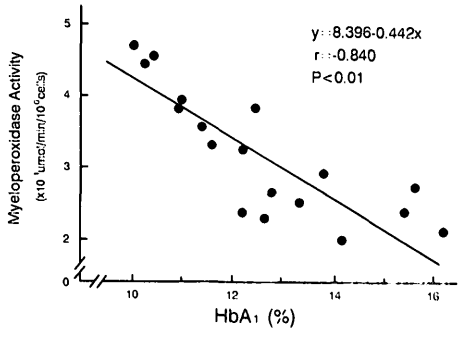

Figure 2—*Correlation between HbA_1 and MPO activity of leukocytes from diabetic patients (n = 18).*

leukocytes. The initial substance of active O_2 generation is O_2^-. Market et al. (8) demonstrated that O_2^- accumulation was reduced in diabetes mellitus. Furthermore, Wieruz-Wysocka et al. (9) reported the reduction of phagocytic, microbicidal function, and O_2^- production in diabetes mellitus (9). Because NADPH is supplied from hexose monophosphate shunt, one of the causes of diminished O_2^- production may be a reduced activity of insulin-dependent enzymes in this shunt due to a lack of peripheral insulin effect.

The production of OCl^- from MPO-H_2O_2-Cl^- system is supposed to be more profoundly involved in the oxidation in the production of LDCL than that of O_2^- from NADPH oxidase system (4). We also found the reduction of leukocytes MPO activity in diabetic subjects. The strikingly linear negative correlation between MPO activity and HbA_1 may support the reduction of MPO activity by the continuation of hyperglycemia. Because there was no difference in the K_m values of MPO, the inhibition of MPO activity may be mediated by an allosteric blockade after glycosylation of the enzyme rather than by a direct blockade of a central portion of the enzyme construction. However, the reduction of only MPO activities cannot explain the increased susceptibility to infections because patients with inborn MPO deficiency are not prone to frequent infection. In conclusion, these data indicate that every step in leukocyte ROI generation was reduced in poorly controlled diabetic patients.

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