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SHORT COMMUNICATION

Pioglitazone reduces monocyte activation in type 2 diabetes

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Abstract Inflammation is involved in the pathophysiologic process of atherosclerosis, a frequent complication of type 2 diabetes. The purpose of our study was to investigate the effect of pioglitazone on systemic inflammatory markers and activation of circulating monocytes in type 2 diabetic patients through the dosage of IL-6. Twenty-four metformin-treated patients, in good glycemic control, were randomized to add pioglitazone for 8 weeks or to continue their previous treatment. Blood samples were collected before and at the end of the study to evaluate: serum levels of high sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6 and leukocyte activation. IL-6 production of circulating monocytes after LPS stimulation was similar at baseline and showed a 54% reduction in pioglitazone-group at 8 weeks (9.1 pg/mL, range 0.0–24.3, $P = 0.04$ vs. baseline) while, in controls, did not change at 8 weeks (16.9 pg/mL, range 1.5–58.8). Treatment with pioglitazone, associated with metformin, showed a reduction of IL-6 monocyte production after their in vitro activation with LPS.

Keywords Inflammation · Pioglitazone · Monocytes

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Introduction and aim

Diabetes mellitus is an independent major cardiovascular risk factor [1] and represents a coronary risk equivalent: this is why diabetic patients should join a secondary prevention program, regardless of their cardiovascular history [2]. Inflammation is involved in the pathophysiologic process of atherosclerosis, above all in diabetic patients. Diabetic plaques usually have a greater lipid core burden, a richer inflammatory component and are more commonly complicated by overlying thrombosis [3, 4]. At the same time, although diabetes is a trigger for vascular inflammation, substantial evidence has shown that low-grade inflammation is an important pathogenetic determinant of type 2 diabetes [5]. A new group of antidiabetic agents, thiazolidinediones (TZDs), like pioglitazone and rosiglitazone, may exhibit anti-inflammatory properties in the vessel wall and reduce inflammatory markers of atherosclerosis, like C reactive protein (CRP) or sCD40L, modulating the cardiovascular risk [6–8]. The mechanisms responsible for their anti-inflammatory effects remain largely unknown. The purpose of our study was to investigate the effect of pioglitazone, a PPARgamma-agonist, on systemic inflammatory markers and activation of circulating monocytes in type 2 diabetic patients through the dosage of IL-6, a cytokine that appears to link local and systemic inflammation.

Materials and methods

Twenty-four metformin-treated patients, in good glycemic control, were randomized to add pioglitazone (45 mg/die) for 8 weeks (Group 2) or to continue their previous treatment (Group 1). Peripheral blood samples were

collected before and at end of the study to evaluate serum levels of high sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6 and leukocyte activation investigated through the monocyte production of IL-6 assessed after whole blood in vitro stimulation with 1 ng/mL of lipopolysaccharide (LPS). High sensitivity CRP was measured with a latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring). The median normal value is 0.08, with 90% of normal value <0.3 mg/dL. IL-6 was measured with a commercial assay kit (Quantikine human IL-6, R and D system). To approximate the condition existing in vivo, we analyzed the effects of LPS in whole blood cultures. Aliquots of 1 mL of heparinized whole blood were placed in sterile 1.5 mL centrifuge tubes and either rapidly processed or placed on a rotator and incubated under sterile conditions at 37°C in atmosphere containing 5% CO_2. The samples receiving the LPS stimulation were treated with 1 ng/mL of LPS (*Escherichia coli* 011:B4; Sigma Chemical Co), which reflects the LPS concentration detected during clinical infections. After 4 h of incubation, samples were removed, placed into ice to terminate the stimulation, and immediately processed. The plasma supernatant was removed and stored at −80°C for further analysis. In each patient were assessed HbA1c, Total Cholesterol, HDL, LDL, Triglycerides and BMI. Statistical analysis: because CRP and IL-6 values do not follow a normal distribution, non parametric tests were used (the Kruskal–Wallis test for comparisons between groups and the Wilcoxon test with the Bonferroni correction for comparisons within groups). The remaining continuous variable were compared by using *t* test for paired and unpaired variables. $P < 0.05$ was considered statistically significant.

Results

Only Group 2 showed a significant reduction of and HbA1c after 8 weeks (7.1 ± 0.8 vs. 6.0 ± 0.7, $P < 0.001$). Serum levels of hs-CRP were similar in both groups (P = ns) at baseline and showed a mild but not significant reduction at 8 weeks in the two groups. IL-6 production of circulating monocytes after LPS stimulation was similar at baseline (Group 1: 18.1 pg/mL, range 0.9–117.7; Group 2: 14.1 pg/mL, range 0.0–100.0; P = ns) and showed a 54% reduction in Group 2 at 8 weeks (9.1 pg/mL, range 0.0–24.3, $P = 0.04$ vs. baseline) while, in Group 1, did not change at 8 weeks (16.9 pg/mL, range 1.5–58.8) (Fig. 1). Therefore, treatment with pioglitazone, associated with metformin, showed a reduction of IL-6 monocyte production after their in vitro activation with LPS.

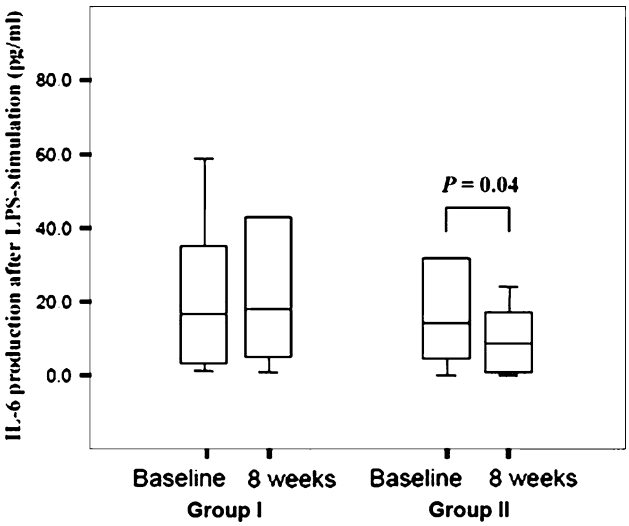

Fig. 1 Monocyte IL-6 production at baseline and after 8 weeks in control (Group I) and treated (Group II) subjects

Discussion and conclusions

Systemic and coronary inflammation play a role in the initiation, progression and precipitation of atherotrombosis [9]; furthermore, diabetes and inflammation could have a synergistic effect in atherosclerosis and this is why it should be available as treatment to modulate the inflammation process in diabetes. A significant increase of macrophage infiltration with overlapped thrombosis is present in coronary specimens from patients with diabetes mellitus. This study demonstrates that treatment of type 2 diabetic patients with PPARgamma-activating thiazolidinediones, together with an improvement of glycemic control, diminishes monocyte production of pro-inflammatory cytokines, as IL-6, that is a significant predictor of future cardiovascular events in healthy subjects as well as in diabetic patients, suggesting a novel anti-inflammatory mechanism for limiting diabetes-associated arterial disease. This finding could be explained as result of the improvement of glicometabolic parameters (reduction of HbA1c) or a double effect of pioglitazone on metabolic pattern and inflammation itself [10].

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