Effect of metformin on fibrinolytic parameters in insulin-treated, type 2 diabetic patients

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SUMMARY
Objective: In the present study, we measured fibrinolytic parameters, including PAI-1 antigen and activity in a group of type 2 diabetic patients in secondary oral anti-diabetic failure treated with insulin alone or with insulin plus metformin.

Research design and methods: 12 type 2 diabetic patients in secondary oral anti-diabetic failure were randomly allocated into two groups receiving insulin alone or insulin plus metformin 1000 mg twice a day; six weeks later, the treatments were swapped over. At the end of each treatment period, blood samples were withdrawn for metabolic and fibrinolytic analysis.

Results: There were no significant differences in fasting blood glucose, fructosamine or fibrinogen; LDL cholesterol, PAI-1 antigen and activity, insulin needs were reduced by the insulin plus metformin regimen (LDL cholesterol: 1.59 ± 0.62 versus 1.28 ± 0.5 mmol/l, PAI-1 antigen: 28.3 ± 17.4 versus 23.9 ± 18 ng/ml, PAI-1 activity: 23.8 ± 9.6 versus 21.9 ± 10 IU/ml, insulin needs: 64 ± 18 versus 52 ± 15 U/day (p < 0.05).

Conclusion: In type 2 diabetic patients with secondary oral treatment failure, insulin alone controlled blood glucose but had no effect on the levels of PAI-1; addition of metformin improved the fibrinolytic parameters.

Key-words: Insulin-treated type 2 diabetic patients - PAI-1 - Metformin.


Effet de la metformine sur les paramètres de la fibrinolyse chez des diabétiques de type 2 insulinosensibles

Objectifs : Les paramètres fibrinolytiques et particulièrement le PAI antigène et le PAI activité ont été suivis dans un groupe de patients diabétiques de type 2 en échec secondaire des antidiabétiques oraux et traités par insuline seule soit seule, soit en association avec la Metformine.

Matériel et méthode : 12 patients diabétiques de type 2 en échec secondaire des antidiabétiques oraux ont été randomisés pour recevoir soit de l’insuline seule, puis de l’insuline plus 1 000 mg de Metformine 2 fois par jour pendant 6 semaines, soit insuline + Metformine dans la première séquence, suivi de l’insuline seule dans la deuxième séquence. A la fin de chacune des séquences thérapeutiques, des prélèvements sanguins ont été obtenus pour l’analyse des paramètres fibrinolytiques.

Résultats : L’équilibre métabolique est resté inchangé, tant au niveau de la glycémie à jeun que de la fructosamine. Le fibrinogène n’a pas été modifié. Le LDL cholestérol, le PAI antigène, le PAI activité et les besoins insuliniques ont été réduits dans la séquence insuline + Metformine (LDL cholestérol 1.59 ± 0.62 versus 1.28 ± 0.5 mmol/l, PAI antigène 28.3 ± 17.4 versus 23.9 ± 18 ng/ml, PAI activité : 23.8 ± 9.6 versus 21.9 ± 10 IU/ml, besoin insulinique 64 ± 18 versus 52 ± 15 U/j (p < 0.05).

Conclusion : Chez les patients diabétiques de type 2 en échec secondaire du traitement oral, l’insulinothérapie seule contrôle le niveau glycémique mais n’a pas d’effet sur les niveaux de PAI antigène et de PAI activité. L’addition de Metformine à l’insuline est susceptible d’améliorer les paramètres de la fibrinolyse, eux-mêmes en rapport avec l’insulinorésistance.

Mots-clés : Diabète type 2 insulino traité - PAI 1 - Metformine.

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Type 2 diabetes is a complex disease associating various pathophysiologic abnormalities with a continuous decline in insulin secretion [1] and a state of insulin-resistance [2]. The decrease in beta cell secretion usually requires insulin replacement therapy after several years of oral anti-diabetic treatment. The difficulty is that although insulin replacement improves blood glucose levels, it does not act on the insulin resistance that is a risk factor for cardiovascular complications [3]; cardiovascular disease (CVD) is the major cause of morbidity and mortality in type 2 diabetic patients. It would thus be expected that therapies improving insulin resistance would help in the management of type 2 diabetic patients. Metformin has been shown to be effective against insulin resistance [4, 5] and metformin has been found to improve fibrinolytic parameters [6, 7] evidenced by a reduction in levels of PAI-1 [8, 9]. However, to our knowledge, the efficacy of insulin plus metformin has not been tested on fibrinolytic activity in type 2 diabetic patients after failure of oral hypoglycemic agents.

In the present study, we measured hemostatic parameters, including PAI-1 antigen and PAI-1 activity in a group of type 2 diabetic patients after failure of oral anti-diabetic agents. The decrease in beta cell secretion usually requires insulin replacement therapy after several years of oral anti-diabetic treatment. The difficulty is that although insulin replacement improves blood glucose levels, it does not act on the insulin resistance that is a risk factor for cardiovascular complications [3]; cardiovascular disease (CVD) is the major cause of morbidity and mortality in type 2 diabetic patients. It would thus be expected that therapies improving insulin resistance would help in the management of type 2 diabetic patients. Metformin has been shown to be effective against insulin resistance [4, 5] and metformin has been found to improve fibrinolytic parameters [6, 7] evidenced by a reduction in levels of PAI-1 [8, 9]. However, to our knowledge, the efficacy of insulin plus metformin has not been tested on fibrinolytic activity in type 2 diabetic patients after failure of oral hypoglycemic agents.

In the present study, we measured hemostatic parameters, including PAI-1 antigen and PAI-1 activity in a group of type 2 diabetic patients after failure of oral anti-diabetic treatment with insulin alone or with insulin plus metformin.

Patients

We studied 12 type 2 diabetic patients. Their age ranged from 60 to 69 years and the duration of their diabetes was 15.4 ± 7.7 years. None of the patients had any history of stroke, coronary artery or heart disease or peripheral vascular disease. Patients with a condition or treatment known to affect hemostatic variables or glucose tolerance were excluded. All the patients were in secondary oral anti-diabetic failure, taking the maximum therapy (15 mg glibenclamide, 2000 mg metformin and 300 mg of a glucosidase inhibitor), with no weight increase and uncontrolled HbA1c. All the patients were therefore candidates for insulin therapy. Metformin and other oral anti-diabetic agents were withdrawn and the patients received twice a day insulin-therapy and were instructed in blood glucose control and insulin dosage. They were treated with two insulin injections a day (a mixture of rapid and slow-acting), one in the morning before breakfast, and another before dinner; target blood glucose was 140-180 mg/dl after breakfast and dinner with a target of 80-120 mg/dl at the end of the afternoon (19 h) and on waking (7 h). After three months of this new treatment they were randomly allocated into two groups, one receiving insulin alone and the other insulin plus metformin 1000 mg twice a day. Six weeks later, the treatments for the two groups were swapped over. During the two periods, diet recommendations and therapeutic advice were unmodified (ACE inhibitors or statins were kept constant if they were taken).

When the patients were receiving insulin plus metformin they were instructed to keep the metformin dosage constant throughout the study and to adjust the insulin dosage if needed.

At the end of each treatment period, blood samples were withdrawn for assay of metabolic and fibrinolytic parameters. Fasting blood glucose, HbA1c, fructosamine, total cholesterol, triglycerides, HDL and LDL cholesterol were determined using routine methods. Blood samples for PAI-1 activity and antigen and fibrinogen were taken in the morning after an overnight fast before breakfast, avoiding venous stasis. Fibrinogen was assayed according to the method described by von Claus and PAI-1 activity (PAI-1 act) was determined using a two-stage indirect enzymatic assay (Biopool Spectrolyse). The results were expressed in IU/ml. PAI-1 antigen (PAI-1 Ag) was measured with an ELISA method using monoclonal antibodies (Immulyse PAI-1 Biopool); only free and latent PAI-1 were detected. The results were expressed in ng/ml.

Clinical parameters: height, weight and abdominal circumference were measured; body composition was monitored by impedancemetry (L’Impulsion, Heronville, France) in a standardized protocol. None of the patients were smokers. The study was approved by the ethical committee of our institution.

Results

No adverse events were recorded during either therapeutic regimen. Blood glucose was monitored successfully and there were no major hypoglycemic events. Body weight was unchanged during the observation period (75.15 ± 9.4 kg at the end of the insulin plus metformin treatment and 75.17 ± 10.2 kg at the end of the insulin treatment). Body composition was not modified with no change in peripheral abdominal circumference.

At the end of each treatment period, there were no significant differences in fasting blood glucose (8.7 ± 2.2 mmol/l versus 9.1 ± 2.1 mmol/l), HbA1c, (8.1 ± 1.2% versus 8.2 ± 0.8%), fructosamine (301 ± 81 µmol/l versus 295 ± 47 µmol/l), triglycerides (1.90 ± 1.37 mmol/l versus 1.75 ± 1.07 mmol/l) or total cholesterol. However, LDL cholesterol was reduced after the treatment with insulin plus metformin (1.59 ± 0.62 mmol/l versus 1.28 ± 0.50 mmol/l, p < 0.05). For the hemostatic parameters: fibrinogen was unchanged but PAI activity and antigen were reduced by the insulin plus metformin regimen (PAI-1 activity: 23.8 ± 9.6 IU/ml versus 21.9 ± 10 IU/ml) (PAI-1 antigen: 28.3 ± 17.4 ng/ml versus 23.9 ± 18 ng/ml) (p < 0.05). Less insulin was required during the metformin ± insulin treatment period (64 ± 18 units/day versus 52 ± 15 units/day) (p < 0.05).
**Table I**

Clinical and biological data in type 2 diabetic patients treated with insulin or insulin + metformin.

<table>
<thead>
<tr>
<th></th>
<th>Insulin</th>
<th>Insulin + Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>75.15 ± 9.4</td>
<td>75.17 ± 10.2</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mmol/l)</td>
<td>8.7 ± 2.2</td>
<td>9.1 ± 2.1</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.1 ± 1.25</td>
<td>8.2 ± 0.8</td>
</tr>
<tr>
<td>Fructosamine (µmol/l)</td>
<td>301 ± 81</td>
<td>295 ± 47</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.90 ± 1.37</td>
<td>1.75 ± 1.07</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.28 ± 2*</td>
<td>4 ± 2.1*</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>159 ± 0.62</td>
<td>128 ± 0.50</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>3.91 ± 0.72*</td>
<td>3.90 ± 0.52*</td>
</tr>
<tr>
<td>PAI-1 activity (IU/ml)</td>
<td>23.8 ± 9.6*</td>
<td>21.3 ± 10*</td>
</tr>
<tr>
<td>PAI-1 antigen (ng/ml)</td>
<td>28.3 ± 17.4*</td>
<td>23.9 ± 18*</td>
</tr>
<tr>
<td>Insulin needs (U/D)</td>
<td>64 ± 18</td>
<td>52 ± 15</td>
</tr>
</tbody>
</table>

* p < 0.05.

**Discussion**

Impaired fibrinolysis increases the risk of cardiovascular disease [10] and favors intravascular deposition of fibrin. The fibrinolytic system depends on competing processes involving circulating activators of plasminogen and inhibitors of the activator. Plasminogen activator inhibitor type 1 (PAI-1) is considered to be the most important inhibitor of fibrinolysis [11]. Several studies have noted high PAI-1 levels in conditions associated with insulin resistance such as type 2 diabetes, but also in non-diabetic offspring of type 2 diabetic patients [12], women with polycystic ovary syndrome [13], non-diabetic obese patients [14], normal hyperinsulinemic patients [15] or patients with high blood pressure [16] who are known to be insulin-resistant.

PAI-1 is synthesized by various cell types including endothelial cells and fat cells from visceral adipose tissue [17, 18].

In the present study we demonstrated that in type 2 diabetic patients with secondary oral treatment failure, insulin therapy alone controlled blood glucose but PAI-1 levels were still high. The elevated levels of PAI-1 in these patients are thought to be a direct consequence of the state of insulin resistance. The addition of metformin to the insulin treatment of those patients improved the fibrinolytic parameters (PAI-1 activity fell from 23.8 ± 9.6 IU/ml to 21.3 ± 10 IU/ml, and antigenicity from 28.3 ± 17.4 ng/ml to 23.9 ± 18 ng/ml), but the level remains higher than controls.

Type 2 diabetes is a complex metabolic disorder characterized by chronic hyperglycemia resulting from target cell resistance to the action of circulating insulin [2] and a quantitative and qualitative deficiency in insulin secretion with respect to that required for normal glycemic control [19]. Additionally, type 2 diabetes is associated with an increased prevalence of hypertension, disorders in lipid metabolism and fibrinolytic activity [20]. All of these may lead to an increased risk of cardiovascular disease.

Currently, type 2 diabetes is managed in a stepwise manner through diet, exercise and for an extended period with combinations of oral anti-diabetic agents such as sulfonylurea, biguanide and more recently thiazolidinedione. However, despite their initial efficacy, blood glucose and Hba1c, tend to rise to levels that necessitate administration of insulin [21-22].

However, type 2 diabetes is not simply a loss of glycemic control as there is also an associated state of insulin resistance, which leads to hyper-triglyceridemia, obesity and abnormalities of fibrinolysis. These in turn may induce the cardiovascular complications found in such patients [3]. In absolute terms, coronary heart disease represents the leading cause of death and justifies therapeutic strategies of glycemic control that also prevent or reduce the progression of vascular complications. It is not yet clear whether stringent control of blood glucose levels reduces the risk of development of CD in patients with type 2 diabetes. For example, in the UKPDS study factors other than blood glucose appeared to play an important role [23].

Some studies suggest that hyperglycemia per se is not the major player in this excess risk, as CD is prevalent in individuals with frank diabetes as well as in those with slight glucose intolerance [15]. Insulin resistance per se is a risk factor for CD and is associated with abnormalities of several coagulation factors, which may enhance the cardiovascular risk. Yudkin found elevated levels of PAI-1 in patients with type 2 diabetes, particularly in those with CD, whereas levels were generally normal in uncomplicated type 1 diabetics [20], PAI-1 is a strong inhibitor of the fibrinolytic system and has been shown to be a predictive factor of myocardial infarction [10].

Although it might be assumed that tight glucose control with insulin therapy would be equally beneficial for type 2 and type 1 diabetic patients, in practice it not easy to maintain normoglycemia in NIDD [23].

Insulin therapy in type 2 diabetes has been shown to affect the lipid profile favorably by inducing anti-atherogenic alterations in lipoprotein composition [24]. It may also reduce the amount of small dense LDL and oxidation and glycosylation of lipoprotein. But insulin has no influence on fibrinolysis and levels of PAI-1. Defective fibrinolysis in diabetic patients might constitute a factor of pathogenic significance for the development of vascular complications. Implicit in such a concept of the cause and effect of an impairment in fibrinolysis is the possibility that stimulation of fibrinolysis by the administration of drugs might delay the development of CD. In the present study, we demonstrated the effect of the association of insulin and metformin on the fibrinolytic system. These findings are in line with those observed with thiazolidinediones [25-27]. However, sulfo-
nylurea appears to have an influence on fibrinolysis [28]. We also demonstrated an improvement in LDL cholesterol level. This has also been described in individuals with upper body obesity [29] and type 2 diabetics [30], but, to our knowledge, has not been reported in insulin-treated type 2 diabetics.

Metformin may thus be maintained in type 2 diabetics treated by insulin after secondary failure of oral anti-diabetic drugs. An additional advantage is that it helps long-term control of body weight [21]. Our results show that maintaining metformin is also beneficial on fibrinolytic activity and LDL-cholesterol. As suggested by the results of the UKPDS [31], both actions would improve the cardio-vascular risk profile of these patients.

References