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Original article

Cytochrome P450 2E1 activity in diabetic and obese patients as assessed by chlorzoxazone hydroxylation

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Summary – Cytochrome P450 2E1 (CYP2E1) is a phase I detoxification enzyme, which is induced by chronic alcohol consumption. It is involved in the activation of numerous carcinogens and in the production of free radicals. As it has previously been shown to be induced in diabetic and obese rats, the aim of this study was to investigate its induction level in poorly-controlled diabetics and in obese patients (Body Mass Index > 30 kg/m^2). CYP2E1 activity was determined in 35 diabetic and 17 obese patients by using the in vivo chlorzoxazone hydroxylation test. Even though the glucidic parameters were highly disturbed (mean fasting glycemia > 7.9 mmol/L, post prandial glycemia > 12.2 mmol/L and fructosamine > 326 μmol/L), CYP2E1 activity was not enhanced either in insulin-dependent diabetics (IDDs, n = 7) nor in non-obese non-insulin-dependent diabetics (NIDDs, n = 15) when compared to controls (n = 42) (0.21 ± 0.03, 0.33 ± 0.03 and 0.30 ± 0.02, respectively, mean ± SEM). However, this activity was lower in IDDs when compared to NIDDs ($P < 0.05$). In obese patients, with (n = 13) or without (n = 17) NIDD mellitus, CYP2E1 activity was increased by a mean of 40% when compared to controls. In addition, positive correlations were found in all subjects (controls or patients, n = 74) between CYP2E1 activity and serum cholesterol (r = 0.42, $P < 0.0001$), triglycerides (r = 0.44, $P < 0.0001$) and BMI (r = 0.36, $P < 0.001$). Accordingly, subjects with cholesterol and/or triglyceride serum levels above 6.4 and 1.8 mmol/L, respectively, displayed a mean increase of 40% of their CYP2E1 activity vs subjects within the above values. It is believed that individuals with increased CYP2E1 activity are more susceptible to the adverse effects of CYP2E1-mediated activation of toxins and carcinogens. © Elsevier, Paris

cytochrome P450 2E1 / chlorzoxazone / diabetes / obesity

INTRODUCTION

Cytochromes P450 belong to a superfamily of enzymes which play an important role in the metabolism of endogenous substrates (steroids, bile acids, fatty acids, etc) or exogenous compounds [27]. One member, cytochrome P450 2E1 (CYP2E1), is of considerable interest because of its role in the activation of many hepatotoxins such as carbon tetrachloride or acetaminophen, or carcinogenic compounds such as nitrosamines [14]. It is also involved in the generation of reactive oxygen species (ROS) leading to oxidative stress [11]. Therefore, individuals with increased CYP2E1 activity would be expected to be at greater risk for the adverse effects of CYP2E1-mediated activation of toxins and carcinogens. CYP2E1 may be induced by a large number of compounds including industrial solvents (acetone, benzene, carbon tetrachloride, etc) or drugs (isoniazid) [22]. However, its most significant role in humans is its adaptative response to high blood ethanol levels with corresponding acceleration of ethanol metabolism, although it is also physiologically involved during the changes in lipid metabolism and ketone utilization which occur during starvation, obesity and diabetes, as reported in rodents [17, 20, 30, 36]. Ketone metabolism by CYP2E1 may be a potential source of in vivo oxygen radical generation and oxidative damage has been shown to play an important role in the pathophysiology of diabetes and diabetic complica-

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Abbreviations: BMI: body mass index; CHZ: chlorzoxazone; CYP2E1: cytochrome P450 2E1; IDDs: insulin-dependent diabetic patients; NIDDs: non-insulin-dependent diabetic patients

Table I. Biological parameters of patients (results are expressed as mean ± SEM).

Patient	*Age (years)*	*Fasting glycemia (mmol/L)*	*Post-prandial glycemia (mmol/L)*	*HbA1c (%)*	*Frutosamine (μmol/L)*	*Cholesterol (mmol/L)*	*Triglycerides (mmol/L)*	*BMI (kg/m²)*	*Duration of disease (years)*
IDDs $n = 7$	31 ± 4	7.90 ± 1	15.8 ± 1.3	8.7 ± 0.6	389 ± 27	4.36 ± 0.37	1.01 ± 0.17	24 ± 0.9	9 ± 2.2
Non-obese NIDDs $n = 15$	49 ± 3	10.90 ± 1.1	15.2 ± 1.6	10.1 ± 0.8	407 ± 38	6.29 ± 0.4	2.09 ± 0.24	26 ± 0.6	6 ± 1
Obese NIDDs $n = 13$	54 ± 2	9.03 ± 1	12.2 ± 1.3	7.91 ± 0.6	326 ± 38	5.92 ± 0.33	2.20 ± 0.30	37 ± 1.5	9.3 ± 2.8
Obeses $n = 17$	40 ± 3	5.10 ± 0.16	8.09 ± 0.8	5.5 ± 0.2	238 ± 10	5.24 ± 0.3	1.51 ± 0.16	39 ± 1.4	14.4 ± 2.8
Normal range of laboratory values		3.5–5		< 6.0	< 285	3.3–6.4	<1.8		

tions [4]. One must remember that extrapolation from rodents to humans is not always possible. For example, a discordance has previously been reported between the effects of fasting in rodents and those observed in humans [28]. Very few studies have reported the levels of CYP2E1 in diabetic and/or obese subjects, mainly due to the difficulty in measuring this enzymatic activity in vivo. Recently, the effectiveness of chlorzoxazone (CHZ) as a CYP2E1 probe has been demonstrated in vitro and in vivo [26, 29]. A non-traumatic approach has been proposed, which calculates the metabolic clearance of CHZ following oral administration. The kinetic parameters of elimination of this drug and its metabolite 6-hydroxyCHZ have been studied and proposed as a reliable marker of CYP2E1 activity in humans [12, 19, 24]. Therefore, the aim of this study was to determine the level of CYP2E1 activity in obese and in insulin- or non-insulin-dependent diabetic patients with poor glycemic control, using the previously described 6-hydroxyCHZ/CHZ blood ratio method [12].

PATIENTS AND METHODS

Patients

33 diabetic patients were included in this study: 7 insulin-dependent diabetics (IDDs), 3 men, 4 women, aged 21–43 years, and 28 non-insulin-dependent diabetics (NIDDs). The latter were divided into 2 groups: non-obese NIDDs, 9 men and 6 women, aged 31–61 years, with a body mass index (BMI) < 30 kg/m² and obese NIDDs, 8 women and 5 men, aged 47–65 years, BMI > 30 kg/m². All the diabetic patiens were hospitalized for poor glycemic control despite treatment which included insulin (0.8 ± 0.15 U/kg) or oral antidiabetic (sulfonylureas and/or biguanides) treatment. In addition, 17 obese patients without diabetes, according to an oral glucose tolerance, participated in this study: 7 men and 10 women, aged 26–59 years, hospitalized in order to comply with a diet corresponding to their disease (BMI: 31 to 50 kg/m²). The subjects were all moderate alcohol drinkers (< 40 g/day) and were not taking medication which would interfere with CYP2E1 activity (acetaminophen, disulfiram, isoniazid). Initial biological parameters are given in *table I*. The control subjects consisted of 42 healthy subjects, 21 men and 21 healthy women, aged 20–50 years, moderate alcohol drinkers (< 40 g/day), taking no medication and with BMI < 25 kg/m², as previously described [25].

Methods

All the subjects were submitted to a chlorzoxazone test as described below to determine CYP2E1 activity. Diabetic and obese patients had additional biological tests related to their pathological state. They all gave their informed consent for the protocol which was approved by the ethical committee of the CHU MORVAN (CCPPRB, Brest, France). The CHZ test was performed 1 or 2 days following the hospitalization of diabetic or obese patients. After a 12 hr fast, subjects were administered a 500 mg pellet of chlorzoxazone (Lemmon Company, Sellersville, USA). A venous blood sample was withdrawn 2 hours thereafter to measure chlorzoxazone (CHZ) and its hydroxylated metabolite, 6-hydroxychlorzoxazone (6-OHCHZ) by high-performance liquid chromatography [23]. The metabolite/parent drug ratio has previously been shown to reflect CYP2E1 activity [12]. All biochemical parameters were determined using a multiparametric routine automat (Synchron Clinical System Myosotis, Beckman Instruments, Brea, CA, USA). Reagents were used according to the recommandations of the manufacturer for determining glycemia, cholesterol and triglycerides (Beckman Instrument, CA, USA), fructosa-

Table II. CYP2E1 activity, glycemia and β-hydroxybutyrate levels in diabetic and/or obese patients. Values represent mean ± SEM. CYP2E1 activity was assessed using the 6-hydroxychlorzoxazone/chlorzoxazone blood concentration ratio (CHZ ratio). Glycemia and β-hydroxybutyrate levels were measured on the sample which was used for the CHZ determination. Differences between all the groups were tested using ANOVA which was followed by post-tests for comparisons between the groups.

Patient	*CHZ ratio*	*Glycemia (mmol/L)*	*β-hydroxybutyrate (μmol/L)*
IDDs $n = 7$	0.21 ± 0.03 [b]	16 ± 2 [d]	165 ± 46 [c]
Non-obese NIDDs $n = 15$	0.33 ± 0.03	10.8 ± 1.3 [c]	163 ± 33 [c]
Obese NIDDs $n = 13$	0.45 ± 0.06 [a]	10.5 ± 1.1 [c]	154 ± 23 [c]
Obese patients $n = 17$	0.38 ± 0.03 [a]	6.53 ± 0.4	88 ± 12
Controls $n = 42$	0.30 ± 0.02	3.5–5	60–140

[a] $P < 0.05$ vs controls; [b] $P < 0.05$ vs non-obese NIDDs; [c] $P < 0.05$ vs obeses; [d] $P < 0.01$ vs obeses.

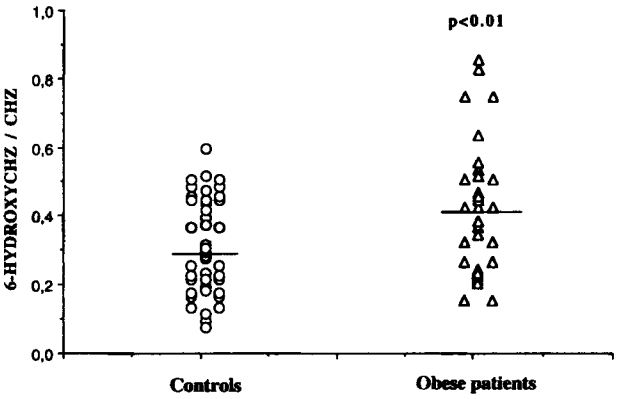

Fig 1. CYP2E1 activity in obese patients. CYP2E1 activity was assessed using the 6-hydroxychlorzoxazone/chlorzoxazone ratio 2 hours after intake of 500 mg chlorzoxazone in 42 healthy controls and 30 diabetic or non-diabetic obeses (BMI > 30 kg/m²). $P < 0.01$ (Student's *t*-test).

mine (Roche Diagnostic System, Somerville, NJ, USA), glycated hemogobin (HbA1c) and β-hydroxybutyrate (Boehringer-Mannheim, Mannheim, D). Fasting and post-prandial glycaemia, fructosamine, HbA1c, fasting cholesterol and triglycerides levels were measured on the first day of hospitalization. In addition, glycemia and β-hydroxybutyrate determinations were carried out on the sample which was used for the CHZ determination, ie, one hour after a light breakfast.

Statistical analysis

Results are expressed as means ± SEM and compared using the Student's *t*-test or by an ANOVA test for multiple comparisons, followed by post-tests for comparisons between all pairs of group means. $P < 0.05$ was taken as the limit of statistical significance. Linear regressions were calculated by the least-squares method and correlation coefficients were determined.

RESULTS

Two hours after oral intake of 500 mg chlorzoxazone (CHZ), the 6-hydroxyCHZ/CHZ serum concentration ratio was assessed in diabetics and/or in obese patients to determine their CYP2E1 activity. Men and women showed similar values for this ratio [25]. As indicated in *table I*, all diabetic patients displayed enhanced fasting glycemia, post-prandial glycemia, glycated hemoglobin or fructosamine, reflecting their poor glycemic control. Obese patients showed mean post-prandial glycemia in the range of glucose intolerance. Cholesterol and triglyceride levels remained at the upper limit of normality for NIDDs.

CYP2E1 activity showed no significant difference in non-obese diabetic patients, either with insulin-dependent-diabetes or with non-insulin-dependent diabetes when compared to controls *(table II)*. It was significantly decreased in IDDs, when compared with non-obese NIDDs. However, as shown in *figure 1*, obese patients, either with or without non-insulin-dependent diabetes, displayed a 40% mean increase of CYP2E1 activity when compared with controls, although it remained in the physiological range (0.41 ± 0.03 vs 0.30 ± 0.02, $P < 0.01$). Glycemia levels and β-hydroxybutyrate levels were higher in diabetic than in obese non-diabetic subjects ($P < 0.05$).

The endogenous factors which regulate CYP2E1 are not well known. A large interindividual variability in CYP2E1 was reported in controls with 6-hydroxyCHZ/CHZ ratio values ranging from 0.12 to 0.50 [25] and it is believed that hormonal status, nutritional status or genetic polymorphism may account for this variability [34]. Until recently, no evidence for a significant relationship between the CYP2E1 enzymatic activity and the described genetic polymorphism has been demonstrated [7, 25]. This is confirmed in the present study (data not shown) and therefore, hormonal or nutritional status may play a more important role. However, attempts to correlate CYP2E1 activity with fasting glycemia, post-prandial glycemia, glycated hemoglobin and fructosamine or even levels of insulin or peptide C (results not shown) were unsuccessful in diabetic as in obese patients. Similarly, no

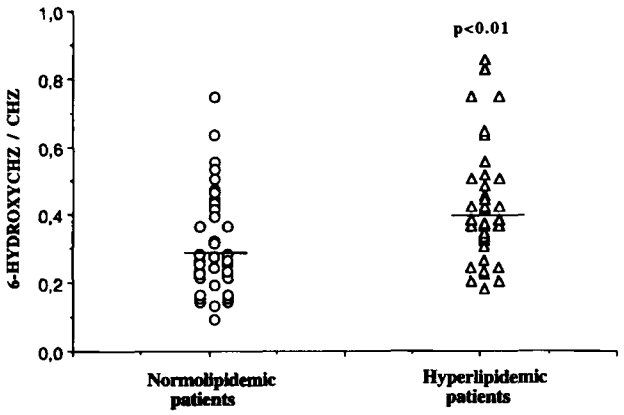

Fig 2. CYP2E1 activity in 'hyperlipidemic' subjects. CYP2E1 activity was assessed using the 6-hydroxychlorzoxazone/chlorzoxazone ratio 2 hours after intake of 500 mg chlorzoxazone in 39 subjects with serum cholesterol < 6.4 mmol/L and triglycerides < 1.8 mmol/L, entitled 'normolipidemic' and 35 subjects with serum cholesterol > 6.4 mmol/L and/or triglycerides > 1.8 mmol/L, entitled 'hyperlipidemic'. $P < 0.01$ (Student's t-test).

correlation was found between CYP2E1 activity and glucose or β-hydroxybutyrate when determined at the time of the CHZ test. Interestingly, significant correlations between CYP2E1 activity and serum cholesterol ($r = 0.42$, $P < 0.0001$) or triglyceride fasting levels ($r = 0.44$, $P < 0.0001$) were found in the whole population (22 controls and 52 diabetic and/or obese patients). Therefore, when subjects were divided into 2 groups, one with cholesterol and/or triglycerides levels above 6.4 and 1.8 mmol/L, respectively, and the other with values beyond this level, a significantly higher CYP2E1 activity was observed in the first group as shown in *figure 2* (0.41 ± 0.03, $n = 35$ vs 0.30 ± 0.02, $n = 39$, respectively, $P < 0.01$). In addition, a significant correlation was also found in this population between CYP2E1 and BMI ($r = 0.36$, $P < 0.001$) but no correlation was found between either cholesterol or triglycerides levels and BMI.

DISCUSSION

The 6-hydroxyCHZ/CHZ concentration ratio was assessed in order to determine if CYP2E1 activity was induced in diabetics and/or in obese patients. This was previously shown to be the case in diabetic or obese rodents [30, 36]. The results obtained in our series of IDDs with poor glycemic control differ from those of Song et al [33] who reported an increase in lymphocyte CYP2E1 in poorly controlled insulin-dependent human diabetics. Accordingly, Goldstein et al [13] reported in IDDs an increased plasma clearance of antipyrine, a marker for P450-dependent hepatic drug metabolism. Antipyrine plasma clearance decreased to that of normal subjects, in diabetic patients whose diabetic control was improved. It is important to note that in these two studies, the patients were younger (mean age, 11 and 15 years, respectively) and the mean fasting glycemia and glycated hemoglobin were twice the upper normal value while in our study, patients were older (mean age 31 years) and their metabolic disorders were less severe (1.5 times the normal values). It has already been demonstrated in animals that the effects of diabetes on P450 are restricted to the period following the onset of the disease, and that induction is less pronounced as the disease progresses [3]. As demonstrated by our data, CYP2E1 activity was not induced after several years of IDD.

CYP2E1 induction could be attributed to hypoinsulinemia as administration of insulin reverses this induction [8, 10]. It was also shown that insulin down-regulates CYP2E1 expression at the mRNA level [9]. As IDDs received insulin as part of their treatment during their hospitalization, this could explain the lower values observed in IDDs when compared to NIDDs receiving oral hypoglycemic agents. Induction of CYP2E1 in diabetes could also be attributed to increased levels of ketones as plasma β-hydroxybutyrate levels were found to correlate with CYP2E1 activity in diabetic rats [1, 5]. Ketosis, which may play a role in the increase of CYP2E1 mRNA [32], appears to be a common feature in CYP2E1 induction which occurs during fasting, diabetes or fat feeding [39]. However, in primary rat culture hepatocytes, Zangar et al [40] recently showed that intracellular levels of fatty acids and ketone bodies did not regulate the expression of CYP2E1. In our study, diabetic patients had higher levels of β-hydroxybutyrate than the non-diabetic obese patients but lower CYP2E1 activity, which also suggests other factors of induction.

In marked contrast to the effect reported in IDDs, Barnett et al [2] showed no changes in CYP 1A, 2B, 2E, 3A, or 4A activities in spontaneously obese-diabetic mice, which exhibit many features reminiscent of human non-insulin-dependent diabetes. Sotaniemi et al [35] reported normal antipyrine clearance (P450-dependent metabolism) in NIDDs in the 36–75 years range. Accordingly, NIDDs displayed CYP2E1 activities similar to those of controls.

However, in our patients, obesity appears to play a more important role than diabetes in modulating CYP2E1 activity. In agreement with the study of O'Shea et al [28], a mean increase of 40% of CYP2E1 activity was found in obese patients, with or without diabetes. This increase is less dramatic than the 100 to 400% observed in alcoholics [24] but one must take into consideration the toxicity of certain compounds

such as acetaminophen or halogenated anesthetics which are bioactivated by CYP2E1. For example, defluorination of both enflurane and sevoflurane, which is CYP2E1-mediated [18], is markedly elevated in obese patients anesthezised with these agents [15] and it has been shown that high fluoride levels could be associated with nephrotoxicity [16]. Hepatic drug metabolism, which requires P450 oxidation, does not appear to change substantially in obese patients [6] although their livers are larger than those of normal-weight control subjects because of an increase in the number and size of parenchymal cells. However, recent reports showed that hepatic CYP2E1 was significantly increased in patients with non-alcoholic liver steatosis [21] or steatohepatitis [37] which occurs in obesity, hyperlipidemia or diabetes. Therefore, CYP2E1 induction appears to be related to steatosis.

Subjects with high levels of cholesterol (> 6.4 mmol/L) or triglycerides (> 1.8 mmol/L) in their plama, also displayed higher mean CYP2E1 levels. The relationship between these parameters and CYP2E1 activity has to be clarified. It was previously shown that endogenous fatty acid are substrates for CYP2E1 [22] and that lipid diets result in CYP2E1 induction at the mRNA level in rodents [32, 38].

In conclusion, this study shows that non-obese diabetic patients, treated either with insulin or oral hypoglycemic drugs for several years, did not display enhanced CYP2E1 activity even though they had severe disturbances in their glycemic balance. Therefore, CYP2E1 does not appear to play an important role in the complications of diabetes. Interestingly, obese patients and individuals with high serum levels of cholesterol (> 6.4 mmol/L) or triglycerides (> 1.8 mmol/L) showed a 40% increase in this activity. It is believed that subjects with high CYP2E1 levels are more susceptible to adverse reactions linked to CYP2E1 bioactivation of some toxins or carcinogens.

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