

SHORT COMMUNICATION

METABOLISM OF LIDOCAINE BY LIVER MICROSOMES FROM STREPTOZOTOCIN-DIABETIC RATS

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Taking into consideration a number of diabetic patients and a paucity of available data on drug pharmacokinetics, it is important to characterise metabolism of drugs, which can be applied in such a group of patients. The aim of the present study was to evaluate metabolism of lidocaine by liver microsomes from streptozotocin-diabetic rats. Liver microsomes were prepared by differential centrifugation and *in vitro* metabolic studies were carried out at linear dependence of the metabolite formation on time, as well as protein and substrate concentrations. The formation rate of monoethylglycinxylydide (MEGX), main lidocaine metabolite on N-deethylation pathway, was evaluated. It was found that both specific and molecular activity of P-450 N-deethylation pathway was significantly reduced in streptozotocin-diabetic animals. The specific activity of cytochrome P-450 was significantly reduced by 69.3% ($p < 0.05$) in diabetic animals in comparison to the controls. Similarly, the molecular activity of the enzyme in streptozotocin-diabetic rat microsomes decreased by 72.5% ($p < 0.05$). The observed changes indicate an impairment of N-deethylation, i.e. a possible decrease in enzymatic activity of CYP3A2 and CYP1A2, which are the major enzymes catalyzing this reaction.

Key words: experimental diabetes, lidocaine metabolism, microsomes

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INTRODUCTION

Diabetes mellitus, a disease with wide prevalence in humans, involves many complications, including micro- and macroangiopathy as well as neuropathy, which in turn lead to increased mortality. In diabetic population, a higher incidence of heart disease, renal failure, cerebrovascular disorders, and neuropathy has been observed. Beside these pathological states, it is believed that possible diabetes-induced alterations in the hepatic biotransformation of pharmaceutical agents could also pose additional health risk because of dangerous side effects due to drug toxicity. Although diabetes is known to cause profound alterations in intracellular metabolism in most tissues, little definitive data are available concerning the effects of diabetes on hepatic drug metabolism [4, 6, 9, 10, 22]. The results of these studies are not univocal and are often contradictory. Some authors suggest that in animals with chemically induced diabetes, the observed changes in drug metabolism may be related to pathological state, but not to an altered metabolic capacity of the liver [21]. However, it was also reported that experimental diabetes was associated with an impairment of some metabolic pathways in the liver [1, 4, 9, 22].

Taking into consideration a number of diabetic patients and a paucity of available data on drug pharmacokinetics, it is important to characterize kinetics of drugs, especially those metabolized in the liver, which can be applied in such a group of patients. A representative of these drugs is lidocaine, whose hepatic metabolism was well documented both in animals and humans. The drug is mostly eliminated *via* biotransformation in the liver on two main pathways, i.e. N-deethylation and hydroxylation with formation of monoethylglycinxylylidide (MEGX) and its metabolite glycinxylylidide (GX) as well as 3- and 4-hydroxylidocaine, respectively [14, 17].

The aim of the study was to evaluate the biotransformation of lidocaine by liver microsomes from streptozotocin-diabetic rats.

MATERIALS and METHODS

Animals

The study was carried out on male Wistar rats (280–380 g) kept under standard laboratory conditions. The animals were randomly divided into two

experimental groups: group I – control animals, and group II – streptozotocin-diabetic rats.

Diabetic rat model

The animals were made diabetic with a single intravenous injection of streptozotocin at 60 mg/kg bw (Sigma, USA). The streptozotocin was dissolved in 0.9% saline containing 0.01 M sodium citrate (pH adjusted to 4.5), and than 1 ml per rat was injected *via* the tail vein within 30 min of its preparation. The diabetic state was confirmed by measurement of nonfasting plasma glucose exceeding 200 mg/dl, evaluated after streptozotocin administration [1, 7]. Glucose concentration was measured using Olympus AU 560 analyzer (Bio-Merieux).

Preparation of liver microsomes

Liver microsomes were prepared by differential centrifugation in 20 mM Tris/KCl buffer (pH = 7.4) including washing with 0.15 M KCl, according to a conventional method. Following microsomes' isolation, a total concentration of cytochrome P-450 was measured by means of Perkin Elmer (Lambda 6 UV/Vis) spectrophotometer, using the method described by Omura and Sato [16]. Total protein content was evaluated according to the method by Lowry et al. [11], using bovine albumin as a standard.

In vitro studies into N-deethylation of lidocaine in rat liver microsomes

The *in vitro* metabolic studies were carried out at linear dependence of the metabolite formation on time as well as on protein and substrate concentrations according to Daniel et al. [5] and Oda et al. [15]. Pooled liver microsomes from control rats were used. Each sample was prepared in duplicate. The optimal conditions of lidocaine N-deethylation were set as follows: time of enzymatic reaction – 7 min, microsomal protein concentration – 0.8 mg of protein per 1 ml, and lidocaine concentration – 70 nmol/ml. Basing on the obtained results, the lidocaine metabolism in liver microsomes was studied at linear dependence of the product formation on time, as well as on protein and substrate concentrations. Microsomes from six control animals and six streptozotocin-diabetic rats were used at this stage of the study. Incubations were carried out in a system containing liver microsomes (ca.

0.4 mg of protein in 1 ml), buffer NaH_2PO_4 (0.1 M, pH 7.4), NADPH (10 mM) and lidocaine at a concentration of 70 nmol/ml for 7 min. at 37°C. Final incubation volume was 0.5 ml. After the incubation, the reaction was stopped by adding 100 μl of methanol and cooling it down to 0°C. The concentration of lidocaine metabolite, MEGX after its extraction from incubation mixture was determined by HPLC method according to Chen et al. [2, 3]. On the basis of these results, a specific and molecular activity of cytochrome P-450 N-deethylation was calculated. Specific activity of the enzyme was defined as a rate of MEGX formation per mg of protein, per minute while molecular activity was a specific activity of the enzyme per 1 mol of the enzyme (cytochrome P-450).

Statistical analysis

The obtained results are presented as means \pm SD. Statistical analysis was performed using the non-parametrical Kolmogorov-Smirnov test, with a p value of < 0.05 considered as statistically significant.

RESULTS and DISCUSSION

In the present study, we determined cytochrome P-450 activity and protein content, and on the basis of these results we evaluated the specific and molecular cytochrome P-450 N-deethylation pathway activity, measured by MEGX formation rate. It was found that both specific and molecular activity of P-450 N-deethylation pathway was significantly reduced in streptozotocin-diabetic animals (Tab. 1). The specific activity of cytochrome P-450 was significantly reduced by 69.3% ($p < 0.05$) in diabetic animals in comparison to the controls. Similarly, the molecular activity of the enzyme in streptozotocin-diabetic rat microsomes decreased by 72.5% ($p < 0.05$).

Streptozotocin-induced diabetes is one of the most popular models of experimental, insulin-dependent diabetes. The period, when the study was carried out, i.e. 10 days from streptozotocin administration, reflects an acute phase of insulin-dependent diabetes, contrary to more remote time points, i.e. 20 days up to 3 months, which is a counterpart of chronic diabetes in humans [19].

Microsomal mixed-function oxidase system, whose major part constitutes cytochrome P-450 plays an important role in oxidation of many drugs and other xenobiotics, e.g. vitamins, toxins, carcinogens as well as endogenous compounds, e.g. prostaglandins, leucotrienes, fatty acids, steroids and many others. Several of many isoforms of cytochrome P-450 are implicated in drug metabolism, mainly CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. These enzymes play an important role in metabolism of drugs routinely used in clinical practice [23]. Expression of these cytochrome P450 isoenzymes can be induced or inhibited by many enzymatic inducers or inhibitors, and also by disease states such as diabetes and hypertension [6, 8, 12, 13]. An increased activity of CYP2E1, CYP4A2 and CYP4A3 in microsomes of streptozotocin-diabetic rats was demonstrated. Contrary, the activity of CYP2C11 in those animals was reduced [18]. Reports on the activity of CYP3A2, a counterpart of human CYP3A4, in rats with experimental diabetes are contradictory. Thummel and Schenkman [20] noted an increase in the enzyme activity, whereas Shimojo et al. [18] demonstrated a decrease in the activity in diabetic rats.

In the present study, male rats were used in the model of experimental diabetes, since Meftah and Skett [12] noted sex differences in the metabolism of lidocaine in the rat. However, the same authors reported that induction of diabetes in the male rat abolished these sex-related differences. The study demonstrated a significant reduction of specific and molecular activity of cytochrome P-450 N-de-

Table 1. Specific and molecular activity of cytochrome P-450 towards N-deethylation of lidocaine in streptozotocin-diabetic rats and in the control animals

Group	Protein (mg/ml)	Cyt. P-450 (nmol/mg protein)	Specific activity (nmol/mg protein/min)	Molecular activity (specific activity/mol cyt. P-450)
Control rats	14.06 \pm 0.64	0.80 \pm 0.05	1.66 \pm 0.20	2.07 \pm 0.19
Diabetic rats	12.50 \pm 1.94	0.92 \pm 0.10	0.51 \pm 0.11*	0.57 \pm 0.15*

* $p < 0.05$

ethylation pathway activity measured as MEGX formation rate in streptozotocin-diabetic rats. The observed changes indicate an impairment of N-deethylation, i.e. a possible decrease in enzymatic activity of CYP3A2 and CYP1A2, which are the major enzymes catalyzing this reaction.

A reduced metabolism of lidocaine may lead to an increase in lidocaine blood concentration, which in turn may precipitate drug-related side effects in diabetic patients. However, as lidocaine metabolism depends on the liver blood flow, *in vivo* studies should complement the present study to elucidate a final effect of diabetes on lidocaine pharmacokinetics.

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