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RESEARCH ARTICLE

A Study on the Optimization of CYP2E1 Enzyme Activity in C57Bl/6 Mouse Brain and Liver

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SUMMARY

In recent years, some studies have shown that CYP2E1 enzyme is associated with many diseases. Determining the activity of this enzyme will help to obtain new clues about several mechanisms in unknown progression of diseases. The purpose of this study is to assess the optimal conditions for CYP2E1 activity. In this context, the optimum conditions (amount of protein, substrate concentration and incubation time) were investigated in C57Bl/6 *mouse brain and liver microsomes. In this study, it was found out that, different reaction conditions are required for this enzyme activities in microsomal brain and liver CYP2E1 of* C57Bl/6 *mice. According to our research results, these optimization studies should be examined for CYP2E1 activity in different tissues of mouse for future investigation.*

Key Words: *Brain, CYP2E1, CYP450, liver, mouse.*

C57Bl/6 Fare Beyin ve Karaciğer Mikrozomlarında CYP2E1 Enzim Aktivitesi Optimizasyon Çalışması

ÖZET

Son yıllarda yapılan araştırmalar, CYP2E1 enzimi ile birçok hastalığın ilişkisi olabileceğine dair veriler ortaya koymuştur. Bu enzim aktivitesinin belirlenmesi, hastalık süreçlerinde bilinmeyen birçok mekanizmaya ışık tutabilir. Bu çalışmanın amacı, CYP2E1 enzim aktivitesi için gerekli optimum koşulları (protein miktarı, substrat konsantrasyonu ve inkübasyon zamanı) araştırmak ve değerlendirmektir. Bu çalışmada, C57Bl/6 *fare beyin ve karaciğer CYP2E1 enzim aktivitesi için farklı reaksiyon koşullarına ihtiyaç duyulduğu gözlenmiştir. Optimizasyon sonuçlarımız, daha kapsamlı araştırmalar için CYP2E1 enzim aktivitesinin farklı fare dokularında da çalışmasının gerekliliğini ortaya koymaktadır.*

Anahtar kelimeler: *Beyin, CYP2E1, CYP450, fare, karaciğer*

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INTRODUCTION

The most important enzyme system of phase I metabolism is cytochrome P450, a superfamily of heme-containing mono-oxygenases and responsible for the metabolism of drugs, xenobiotics, environmental compounds, carcinogens and several endogenic compounds (1,2). These enzymes are abundant in the liver, it also exists in some extrahepatic tissues such as kidney, lung, intestine and brain (4). Total CYP levels in the brain are low and approximately 0,5-2% of those in the liver (2). CYPs are distributed homogeneously in the liver whereas each of CYP450 isoenzymes is located according to their specific localization in the brain (3). Therefore, it is difficult to determine the enzymatic activity of CYPs in the brain.

CYP2E1 is involved in the detoxification-activation reactions of various endogenous and exogenous compounds. Besides a role in the ethanol metabolism, CYP2E1 catalyses the metabolism and activation of drugs (e.g. halothane, enflurane, sevoflurane, acetaminophen), endogenous substrates (e.g fatty acids, ketones), several procarcinogens (e.g. nitrosamines, benzene) and halogenated hydrocarbons (e.g. carbon tetrachloride, 1,1,1-trichlorethane, methlychloride, vinylchloride) (4-7).

It was the first identified in the microsomal ethanol-inducing system (MEOS) (8). This P450 isoform has been observed in many organism such as rabbit, rat, mice and human. CYP2E1 is one of the preserved forms in CYP450 enzymes family and its catalytic activities are quite similar among the species. It is difficult to find a common feature of substances that are metabolized by CYP2E1 but in general, small, hydrophobic and under 70 kDa molecular weight compounds (more

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than 70 different chemicals) are suitable to be catalyzed by CYP2E1 (9,10).

Among CYP450 enzymes, CYP2E1 has a remarkable effect on toxicity. It can also generate reactive oxygen species during its catalytic circle which contributes to oxidative stres and causing lipid peroxidation, protein inactivation and DNA damage (11). The substrates of CYP2E1 have become more toxic and carcinogenic substances via its catalytic activity (16). Thus, it is thought to have a role in progression of diseases. On the other hand, CYP2E1 levels in liver increases under some pathophysiological conditions such as alcohol consumption (12), diabetes (13), obesity (14) and starvation (15) in animals and humans.

As other CYPs, CYP2E1 levels are low in the brain when compared to the liver. But, most of the studies have indicated that activities of this enzyme can varied in the presence of inducer or inhibitor. Some regions and cells CYP2E1 levels in the brain are expressed as high as those in the liver. In experimental conditions, recent studies have shown inducer-dependent induction of brain CYP2E1 catalytic activity to be %125 of the liver both in rats and humans (17-22).

Its catalytic activity can be measured with N-nitrosodimethlyamine (NDMA), p-Nitrophenol (pNP) and Chlorzoxazone (CLZ) in vitro (23). Based on previous studies, pNP was selected substrate of CYP2E1. This chemical has been indicated the activity of CYP2E1 in microsomal samples containing. pNP is a phenolic substance and a solution of the phenolate salt is yellow. CYP2E1 hydroxylates pNP at the 2 position to generate 4-Nitrocatechol (4NC) (24).

Activities of brain and hepatic CYPs are of key relevance for the susceptibility to system diseases and treatment outcomes. CYP2E1 is known to be expressed in mouse liver, as well as in various mouse brain regions. In this study, the catalytic activity of CYP2E1 can be estimated in C57Bl/6 brain and liver tissues, using p-nitrophenol metabolism. Determination of the CYP2E1 catalytic activity in brain and liver may be important from several toxicological points of view.

MATERIALS AND METHODS

Chemicals

Folin-ciocalteu reagent, Glucose-6-phosphate, Glucose-6-phosphate dehydrogenase, Nicotineamide adenine dinucleotide phosphate, sodium salt ($NADP^+$), perchloride acid, p-nitrophenol, sodium hydroxide were purchased from Sigma-Aldrich (USA). All reagents were of analytical grade.

Animals

All procedures used in this study were approved by the Ankara University Animal Care and Use Committee. C57Bl/6 (20-25 g, 12 weeks, male) mice were used throughout the experiments. Mice were provided a week ago, because travel changes the brain chemicals (25). The animals were housed 4 per cage in an animal house under standart conditions (12-h light-dark cycle, room temperature; 22-25 °C, optimum humidity) and fed standart pellet and water ad libitum. The animals were sacrificed via cervical dislocation. Brain and liver tissues were carefully dissected and immediately stored at -80 °C.

Preparation of Microsomes

The mouse brain and liver tissues were homogenized (%1,15 KCI (w/v)) in ice-cold condition, and centrifuged for 25 min. at 10.000 g. Centrifugation of the 10.000 g supernatants at 108.000 g. The pellets of ultracentrifugation were homogenized with %20 glycerol and were stored at -80 °C until use.

Determination of Protein Concentration

Microsomal brain and liver proteins were determined by the method of Lowry et. al., using bovine serum albumin as a standard (26).

Determination of pNP-hydroxylase Activity

Microsomal CYP2E1 (p-Nitrophenol-2-hydroxylase) activity was assayed as described by Reinke and Moyer (27). This enzyme activity was determined with CYP2E1 hydroxylates pNP, its 2-hydroxylation to form of 4-Nitrocatechol was measured spectrophotometrically.

Reaction Medium

1 ml of reaction medium contains microsomal protein, as a substrate pNP, 400 mM pH 6.8 tris buffer, 100 mM glucose-6-phosphate, as a cofactor 20 mM $NADP^+$, 100 mM $MgCI_2$, 400 mM pH 7.8 potassium phosphate buffer and 500 U/0.15 ml glucose-6-phosphate dehydrogenase.

Procedure

The reactiom medium (tris buffer, pNP and microsomal protein, respectively) was added to the each test tube. Test tubes containing all substance in the reaction medium were taken in a shaking water bath at 37 °C. Reaction was initiated with the addition of NADPH generating system to each test tube. The samples incubated at 37 °C for 30 min. in a shaking water bath. At the end of the incubation, each reaction was stopped by the addition of 500 µl of 0,75 N (v/v) perchloride acid. Centrifuged for 45 min. at 5 000 g to remove denatured proteins in tubes. After centrifugation, 1 ml of supernatant was taken into another tube. 250 µl of 2 M NaOH was added to complete ionization of 4-Nitrocatechol which formed in the reaction. After the color change

observed in the tubes, centrifuged 5 000 g for 15 min. for the removal of NaOH precipitates. Immediately, after the last centrifugation, the absorbance values were read at a wavelenght of 546 nm spectrophotometrically.

RESULTS AND DISCUSSION

In this study, optimum conditions for CYP2E1 activity in C57Bl/6 mouse liver and brain microsomes were investigated with respect to protein and p-nitrophenol concentration and incubation time.

Effect of Protein Amount on CYP2E1 Activity

The effect of protein amount on CYP2E1 activity in brain was examined in 1 ml incubation medium by the addition of 1.0 mg, 2.0 mg, 4.0 mg and 6.0 mg protein. Increasing CYP2E1 activity was observed to be linear in the range of 1.0-4.0 mg protein. Maximum activity of CYP2E1 was measured at 4.0 mg protein in the brain. While examining the effect of protein amount on hepatic CYP2E1 activity in mice, in the incubation medium was studied by the addition of 1.0 mg, 2.0 mg, 3.0 mg and 4.0 mg protein. Maximum activity was observed at 3.0 mg (Table 1, Figure 1).

Table 1. Results of the optimization of protein amount on CYP2E1 activity in C57Bl/6 brain and liver*.

	CYP2E1 Enzyme Activity (nmol 4-nitrocatechol/min.)	
Amount of Protein (mg)	Brain	Liver
1.0 mg	0,024 ± 0,004	0,121 ± 0,008
2.0 mg	0,050 ± 0,007	0,241 ± 0,014
3.0 mg	-	0,331 ± 0,011
4.0 mg	0,116 ± 0,008	0,134 ± 0,016
6.0 mg	0,050 ± 0,005	-

* All the values are mean ± SEM of 3 experiments. Each experiment was conducted dublicate.

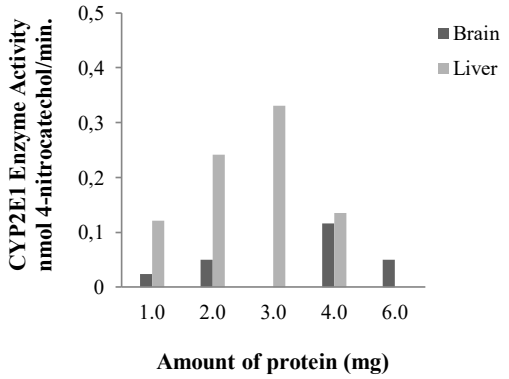

Figure 1. Effect of protein amount on CYP2E1 activity in C57Bl/6 mouse brain and liver.

Effect of Incubation Time on CYP2E1 Activity

The effect of incubation time on CYP2E1 activity in brain and liver was tested with different incubation time between 10-60 min. and maximum activities were observed at 30 minutes in both brain and liver microsomes (Table 2, Figure 2).

Table 2. Results of the optimization of incubation time on CYP2E1 activity in C57Bl/6 brain and liver*.

	CYP2E1 Enzyme Activity (nmol 4-nitrocatechol/mg)	
Incubation Time (min.)	Brain	Liver
10 min.	0,222 ± 0,065	1,93 ± 0,206
20 min.	0,484 ± 0,050	2,74 ± 0,180
30 min.	0,800 ± 0,089	4,08 ± 0,191
60 min.	0,406 ± 0,099	2,43 ± 0,262

* All the values are mean ± SEM of 3 experiments. Each experiment was conducted dublicate.

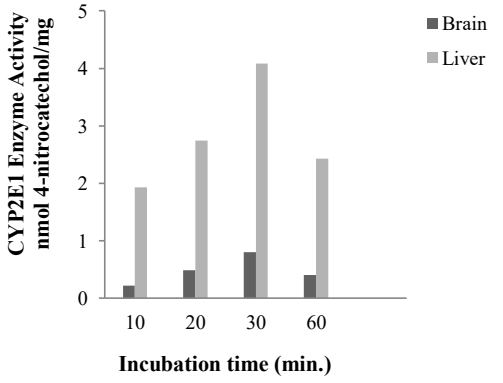

Figure 2. Effect of incubation time on CYP2E1 activity in C57Bl/6 brain and liver.

Effect of Substrate Concentration on CYP2E1 Activity

The effect of pNP concentration on CYP2E1 activity in brain was examined in 1 ml reaction medium by the addition of 62,5 µM, 125 µM, 187,5 µM and 250 µM of pNP. Increasing CYP2E1 activity was observed to be linear in the range of 62,5 µM-125 µM of pNP and maximum activity was observed at 125 µM of pNP in brain. The effect of pNP concentration on CYP2E1 activity in liver was examined in 1 ml reaction medium by the addition of 25 µM, 62,5 µM, 125 µM and 250 µM of pNP and maximum activity was observed at 125 µM of pNP in liver (Table 3, Figure 3). Some studies have reported that CYP2E1 activity was inhibited at concentrations higher than 200 µM of pNP (10,28,29) and these studies lends support to our findings.

Table 3. Results of the optimization of substrate concentration on CYP2E1 activity in C57Bl/6 brain and liver*.

	CYP2E1 Enzyme Activity (nmol 4-nitrocatechol/mg/min)	
p-Nitrophenol Concentration (µM)	Brain	Liver
25 µM	-	0,045 ± 0,005
62,5 µM	0,008 ± 0,001	0,080 ± 0,006
125 µM	0,028 ± 0,001	0,150 ± 0,004
187,5 µM	0,014 ± 0,002	-
250 µM	0,006 ± 0,002	0,085 ± 0,008

* All the values are mean ± SEM of 3 experiments. Each experiment was conducted dublicate.

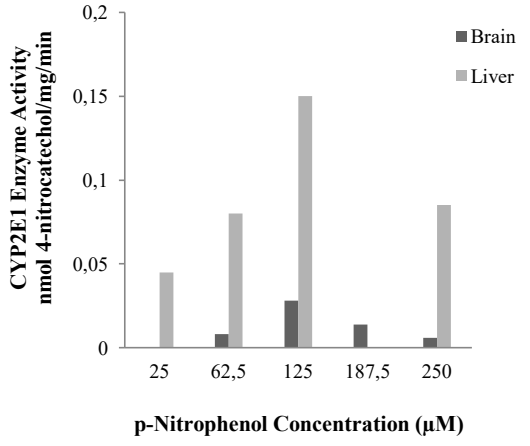

Figure 3. Effect of substrate concentration on CYP2E1 activity in C57Bl/6 brain and liver.

Consequently, in this optimization study it is determined that different reaction conditions are required for CYP2E1 activities in mouse brain and liver microsomes. Furthermore, this study was performed on C57Bl/6 mice which are quite sensitive species. The present results may help to understanding of the functional roles of brain and liver CYP2E1 in prevention and treatment of many diseases.

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