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# CHANGES IN THE ACTIVITY OF SOME DRUG METABOLIZING ENZYME SYSTEMS AND CYTOCHROME P450 QUANTITY AFTER MULTIPLE FLUOXETINE ADMINISTRATION IN RATS

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**Summary.** Fluoxetine is one of the most widely used selective serotonin reuptake inhibitors (SSRIs) in psychiatry. It can be used alone and in combination with other drugs. These combinations can result in adverse drug effects, connected with interactions on metabolic level. In our study, we investigated the influence of fluoxetine after multiple administration on total cytochrome P450 quantity and on drug metabolizing enzyme systems activity – ethylmorphine N-demethylase (EMND) and aniline hydroxylase (AH). Microsomes were prepared by two different methods – ultracentrifugation and low speed centrifugation following sedimentation of the microsomal membranes in the presence of calcium ions. We found no statistically significant difference in results of total cytochrome P450 level between microsomes, prepared by both methods. As a result of the experiment, we found that after multiple administrations fluoxetine, like phenobarbital, increases significantly the level of total cytochrome P450 and the activity of EMND and AH.

**Key words:** rat microsomes, fluoxetine, cytochrome P450, ethylmorphine N-demethylase, aniline hydroxilase

# ПРОМЕНИ В АКТИВНОСТТА НА НЯКОИ ЛЕКАРСТВОМЕТАБОЛИЗИРАЩИ ЕНЗИМНИ СИСТЕМИ И КОЛИЧЕСТВОТО НА ЦИТОХРОМ Р450 СЛЕД МНОГОКРАТНО ПРИЛАГАНЕ НА FLUOXETINE ПРИ ПЛЪХОВЕ

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**Резюме.** Флуоксетинът е един от най-често използваните в психиатрията селективни инхибитори на обратното поемане на серотонин (SSRIs). Той се прилага както самостоятелно, така и в комбинация с други лекарства, което може да доведе до нежелани ефекти в резултат на метаболитни взаимодействия. Целта на настоящото изследване е да се проучи влиянието на флуоксетин, въведен многократно, върху нивото на цитохром Р450 и активността на някои лекарство-метаболизиращи ензими – етилморфин N-деметилаза (EMND) и анилин хидроксилаза (АН), в черния дроб на плъх. Микрозомите са получени по два метода – чрез ултрацентрофугиране и чрез калциева агрегация. Ние установяваме, че резултатите за количеството на цитохром Р450 в микрозомите получени по двата метода, нямат статистически значима разлика. Флуоксетинът, въведен многократно, повишава статистически значимо количеството на цитохром Р450, подобно на фенобарбитала, както и активността на EMND и АН.

**Ключови думи:** микрозоми при плъх, флуоксетин, циохром Р450, етилморфин N-деметилаза, анилин хидроксилаза

## Introduction

Selective serotonin reuptake inhibitors (SSRIs) are used alone and in combination with other medications (phenytoin, resperidone, clozapin, diazepam, alprazolam, propranolol, claritromycine) in the treatment of a variety of psychiatric disorders. Such combinations create the potential for pharmacokinetic interactions by affecting the activity of the cytochrome P450 (CYP450). Naranjo et al. find that as a potent inhibitor of CYP2D6, fluoxetine can increase serum concentrations of neuroleptics, antidepressants and numerous case reports have documented adverse events; the drug also inhibits CYP3A and CYP2C19, increasing serum concentrations of

some benzodiazepines [11]. In 1998 Baker et al., find that CYP2D6 catalyzes the N-demethylation of fluoxetine to produce norfluoxetine, which is an active metabolite with strong serotonin reuptake inhibition. Norfluoxetine may be involved with both the therapeutic and adverse effects associated with fluoxetine [2]. Later Margolis et al., working on the metabolism of fluoxetine find that fluoxetine N-demethylase activity may be catalyzed not only by CYP2D6 but also by others – CYP1A2, -2B6, -2C9, -2C19, -3A4 and -3A5 [9].

In our study, we compare total quantity of cytochrome P450 in microsomes, prepared by two different methods. We investigate fluoxetine effect after multiple administrations on cytochrome P450 quantity and on EMND and AH activity in rat microsomes. Effects of fluoxetine on total cytochrome P450 are compared with phenobarbital effects – a well known inducer of CYP2B1, 2C6 and 3A in rats [3].

## Materials and methods

*Animals*

The experiments are carried out on male Wistar rats (200-250 g) keep under standard laboratory conditions. Animals are purchased from the National Breeding Center, Sofia, Bulgaria. All performed procedures are approved by the Institutional Animal Care Committee and are in accordance with the European Union Guidelines for animal experimentation.

*Experimental procedure*

Rats are treated 8 days with 10 mg/kg i.p. fluoxetine [10] and 4 days with 75 mg/kg phenobarbital [12].

*Microsomes are prepared by two different methods, described by Gibson & Skett [7]*

– Preparation of microsomes using ultracentrifugation. The liver is homogenated in 0.1M TRIS-potassium-phosphate buffer (pH = 7.5). The homogenate is centrifuged for 30 min at 9000 xg, and the supernatant is centrifuged at 105 000 xg for 1 hour (4°C). Microsomes are re-suspended in 20% glycerol solution of 0.1M potassium-phosphate buffer (pH = 7.4).

– Preparation of microsomes using low speed centrifugation and calcium aggregation, with modifications. The liver homogenate, prepared in 0.25M saccharose, is centrifuged for 30 min at 9000 xg. Calcium chloride is added to the supernatant and the mixture is gently shaken on ice. The resulting suspension is centrifuged at 24 000 xg for 45 min. Microsomes are re-suspended in 0.1M TRIS buffer (pH = 7.4).

*Measurement of cytochrome P450 quantity*

Total cytochrome P450 protein in liver microsomes is assayed by carbon monoxide difference spectrum after reduction with sodium dithionite by the method of Gibson & Skett [7].

The protein content of each microsomal preparation is determined by the method of Lowry [8].

*Statistical analysis*

Statistical analysis is performed by applying the Student's *t*-test, with $P < 0.05$ considered statistically significant. All results (n = 12) are expressed as mean ± SD.

*Results*

The total quantity of cytochrome P450 in rat liver microsomes prepared by ultracentrifugation and by low speed centrifugation, following sedimentation of the microsomal membranes in the presence of calcium ions is shown in table 1.

***Table 1.*** *Level of cytochrome P450 in microsomes, prepared by two different methods*

| Group | Total quantity of cytochrome P450 | |
|---|---|---|
| | Ultracentrifugation | Low speed centrifugation, followed by calcium aggregation |
| Control | 0.208 ± 0.01 | 0.212 ± 0.02 |

There are no statistically significant differences between both methods for preparation of microsomes in results of cytochrome P450 total quantity obtained from rat liver.

The effects of fluoxetine on the level of cytochrome P450 compared to effect of phenobarbital in microsomes are shown in table 2.

***Table 2.*** *Fluoxetine effect on cytochrome P450 total quantity in microsomes, prepared by low speed centrifugation*

| Group | Total quantity of cytochrome P450 | Effect vs control |
|---|---|---|
| Control | 0.212 ± 0.02 | 100 |
| Phenobarbital | 0.519 ± 0.03** | ↑ 145% |
| Fluoxetine 10 mg/kg i.p. 8 days | 0.459 ± 0.04** | ↑ 117% |

**P < 0.01 versus control

Quantity of cytochrome P450 in rats treated with fluoxetine 8 days shows a statistically significant incease, compared to the control by 117%. Phenobarbital increases total quantity of cyto-

chrome P450 statistically significant by 145%, compared to the control. Both of are inducers, but fluoxetine is weaker by 12%.

***Table 3.*** *Effect of fluoxetine on the ethylmorphin-N-demethylase activity*

| Group | Activity of EMND | Effect vs control |
|---|---|---|
| Control | 0.384 ± 0.02 | 100 |
| Fluoxetine 10 mg/kg i.p. 8 days | 0.444 ± 0.03* | ↑ 16% |

*P < 0.05 versus control

On table 3, there are shown effects of fluoxetine on ethylmorphin-N-demethylase (EMND) activity. Fluoxetine exhibits statistically significant increased EMND activity by 16%.

***Table 4.*** *Effect of fluoxetine on aniline hydroxilase (AH) activity*

| Group | Activity of AH | Effect vs control |
|---|---|---|
| Control | 0.128 ± 0.01 | 100 |
| Fluoxetine 10 mg/kg i.p. 8 days | 0.251 ± 0.01** | ↑ 96% |

** P < 0.01 versus control

Effect of fluoxetine on aniline hydroxilase activity is shown in table 4. Fluoxetine increases statistically significant this enzyme activity by 96%, compared to the control.

## Discussion

In this experiment, we compare total quantity of cytochrome P450 in microsomes, prepared by two different methods – ultracentrifugation and low speed centrifugation, following by microsomal membrane sedimentation in the presence of calcium ions. We find no difference in total cytochrome P450 quantity in microsomes, prepare by above methods, wherefore bouth could be equally used. These data are supported by the study of Ravindranath & Anandatheerthavarada [13]. The authors find that brain microsomes, prepared by ultracentrifugation, lose rapidly cytochrome P450 activity. For this reason, they use calcium aggregation method for preparing brain microsomes.

During our experiments, fluoxetine induces statistically significant AH activity – 96% – compared to control. It is known that aniline is substrate of CYP2E1. CYP2E1 in rats is an available model to evaluation liver drug metabolism and induction in microsomes. The above isoform is quite well conserved and thus extrapolation between rats and human is performed quite well [15].

In EMND, fluoxetine induces its activity by 16%, compared to control. EMND is a reliable indicator of induced rat hepatic cytochrome P450 CYP3A activity [1]. CYP3A is the most important isoform involved in the metabolism of xenobiotics in all species [15].

We suggest that fluoxetine effects on AH and EMND activity are important for possible drug interaction on metabolic level.

Our results demonstrate that in rats, treated with fluoxetine, level of total cytochrome P450 shows an increases of 117%, compared to the control, while phenobarbital, compared to the control, induces cytochrome P450 by 145%. The increased total cytochrome P450 quantity by fluoxetine is 12% less than that of phenobarbital.

Literature data about fluoxetine inhibitory and inducting effects are controversial. In 2001, Daniel et al. found that fluoxetine *in vitro* has inhibitory effect on CYP2B, CYP3A and CYP2E1 in rat and human microsomess [4]. Later they report that *in vitro* fluoxetine inhibits directly CYP2C6, but *in vivo* during two weeks of treatment in rats it induces CYP2C6 [5]. It is known that CYP2C9 is one of the isoformes catalyzing fluoxetine N-demethylase activity in human [9]. Gamble et al. report that CYP2C6 in rats is orthologue to CYP2C9 in human [6].

These results about fluoxetine effects may be of physiological, pharmacological and toxicological importance and require further investigations for eventual metabolic drug interactions.

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