

1-1-1999

Characterization and Structure-Function Studies of Human Liver Flavin Monooxygenase Isozyme 3 (FM03)

ORHAN ADALI

RICHARD M. PHILPOT

Follow this and additional works at: <https://journals.tubitak.gov.tr/zoology>



Part of the [Zoology Commons](#)

Recommended Citation

ADALI, ORHAN and PHILPOT, RICHARD M. (1999) "Characterization and Structure-Function Studies of Human Liver Flavin Monooxygenase Isozyme 3 (FM03)," *Turkish Journal of Zoology*. Vol. 23: No. 1, Article 5. Available at: <https://journals.tubitak.gov.tr/zoology/vol23/iss1/5>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Zoology by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Characterization and Structure-Function Studies of Human Liver Flavin Monooxygenase Isozyme 3 (FM03)

Orhan ADALI

Middle East Technical University, Dept. of Biology, 06531 Ankara-TURKEY

Richard M. PHILPOT

National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, 27709 NC-USA

Received: 12.01.1998

Abstract: The activity of the flavin-containing monooxygenase (FMO EC 1.14.13.8) can be modulated by a number of nitrogen-containing compounds in a manner that is both isoform and effector-dependent. We showed that the direction (activation or inhibition) and extent of modulation can also be dependent on substrate concentration. The native human liver FM03 and arg 433 mutant FM03 catalyze the methimazole reaction with similar K_m values. However, the mutant FM03-catalyzed reaction was affected differently by high concentration of imipramine, imipramine causing inhibition of activity. Our results suggest that the response of FM03 to imipramine involves a distribution of compounds between two sites which is regulated by structural features.

Key Words: Flavin Monooxygenases (FMOs), Human, FM03 isozyme, Imipramine, liver.

İnsan Karaciğer Flavin Monooksijenaz İzozim 3 (FM03)'ün Karakterizasyonu ve Yapı-Fonksiyon Çalışmaları

Özet: Flavin içeren monooksijenaz (FMO) enzim aktivitesi hem izozim hem de efektöre bağlı olarak nitrojen bulunduran maddeler tarafından değiştirilebilmektedir. Aktivite değişikliğinin yönü (aktivasyon veya inhibisyon) ve şiddetinin substrat konsantrasyonuna da bağlı olabildiği gösterilmiştir. İnsan karaciğer FM03'ün doğal formu ve arjinin 433 mutant FM03, methimazole reaksiyonunu benzer K_m değerleri ile katalize ettiler. Bununla beraber, mutant FM03 tarafından katalize edilen reaksiyon, yüksek konsantrasyonlu imipramin tarafından farklı şekilde etkilendi, imipramin aktivitenin inhibe olmasına sebep oldu. Sonuçlarımız gösterdi ki, FM03'ün imipramine cevabı, yapısal özelliklere ve imipraminin iki bölge arasındaki dağılımına dayanmaktadır.

Anahtar Sözcükler: Flavin Monooksijenazlar (FMOs), İnsan, FM03 İzozimi, Imipramin, karaciğer.

Introduction

NADPH and oxygen-dependent mammalian microsomal flavin-containing monooxygenases (FMOs) oxidize a large number of xenobiotics, including numerous drugs, neurotoxins, pesticides and some endogenous molecules at nucleophilic nitrogen, sulfur and phosphorus atoms (1-6). The ability of FMO to oxidize a variety of xenobiotics is important in both activation and detoxication processes. An inherited defect in the FMO-catalyzed N-oxidation of the dietary-derived amine, trimethylamine (TMA), results in the disorder trimethylaminuria, also known as fish odor syndrome (7, 8). The deficiency of one of the FMO isozymes, which is suggested to be FM03, is responsible for this disease. Affected individuals are unable to metabolize TMA to the more polar and non-odorous N-oxide and excrete the malodorous free amine in their urine, breath and sweat.

Recent studies have shown that FMO gene family consists of at least five members that are expressed in a species-and tissue-dependent manner (9, 10). In contrast to the livers of many animals such as rats, pigs and rabbits, in which FM01 is the dominant hepatic isoform, the human liver contains FM03 (GenBank accession no. M83772) as a major isozyme. Based on the results of catalytic, immunochemical and expression studies, FM03 makes the greatest contribution to FMO-mediated drug metabolism in the adult human liver (11-14). FM03 has been cloned from humans (12) and rabbits (15) and FM03 orthologs have been isolated from macaque, rabbit and rat livers (16-18).

Since native human FM03 has not been purified and characterized yet, catalytic studies conducted with membrane preparations from *Escherichia coli* expression systems are beginning to identify substrates for human

FMO3. Activation and inhibition studies are now under investigation to elucidate the structure-function relation of human FMO3 using the expressed protein. Ziegler (1) described the activation as an increase in metabolism caused either by adding modulators or substrate self-activation.

This study was undertaken to investigate the molecular basis for modulation of FMO3-catalyzed activity by tricyclic antidepressant imipramine and antipsychotic chlorpromazine. For this purpose, the effects of these drugs on recombinant FMO3 catalyzed substrate, methimazole, were studied and the results were compared with those of mutant FMO3 obtained using site-directed mutagenesis to elucidate the structure-function relation of human FMO3 isozyme.

Materials and Methods

Cloning and sequencing of Human FMO3-Human FMO3 was cloned from a human cDNA library constructed using hepatic mRNA isolated from the liver of the adult male, as described previously (15). The library was screened with a 620-base pair 5'-fragment (EcoRI) of the cDNA for rabbit FMO3 (15) random labelled (19) with [α -³²P] dCTP (Boehringer Mannheim). Oligonucleotide primers were used to obtain the sequence of FMO3 from both strands of full-length clones. Double-stranded plasmid DNA was isolated and cDNA inserts end sequenced (Sequenase Version 2.0, U.S. Biochemical Corp., Cleveland, OH) by the dideoxy chain termination method (20). Sequencing protocol was simply based on the denaturation of dsDNA, the annealing of primers to isolated template DNA, the synthesis of a DNA strand terminated by the incorporation of a nucleotide analog (ddNTPs), and the electrophoretic separation of DNA fragments of different lengths.

Expression of FMO3 and FMO3 mutants. *E. coli* cells were transformed with native recombinant and mutant human FMO3 in pJL2 vector. A single colony was grown at 37°C, 250 rpm, in LB medium containing ampicillin (50 µg/ml) to an absorbance of 0.4-0.5 at 600 nm and then overnight at 30°C at 150 rpm in the presence of 1 mM Isopropyl β -D-thio-galactopyranoside (IPTG) to induce expression of recombinant proteins. Cells were harvested by centrifugation at 2,500xg for 5 min at 4°C and resuspended in 10 ml of ice-cold lysis buffer (100 mM KCl, 50mM potassium phosphate, pH 7.4, 1 mM EDTA) containing 1 mg/ml lysozyme. Cells were digested by incubation on ice for 30 min with occasional inversions and then centrifuged (3,00 x g for 30 min) and resuspended in 2 ml ice-cold lysis buffer. Cells were

further lysed by sonicating five times for 30 seconds at full power in ice water bath with 30 seconds cooling between bursts. Cell debris was removed by spinning the solution at 5,000 x g for 12 min. Membrane fragments were collected from the supernatant by centrifugation at 110, 000 x g for 30 min. The pellet was washed in ground-glass homogenizer using 3 ml lysis buffer and resedimented (110, 000 x g for 30 min). The final membrane fraction was suspended in 50 mM potassium phosphate buffer, pH 7.4, containing 20 % glycerol and 1 mM EDTA. Samples were stored at -70°C for characterization of FMO3 protein.

Mutagenesis of FMO3-Point mutation at base position 1282 (base numbered starting with the first base of the start codon) in the recombinant DNA encoding human FMO3 was accomplished by site-directed mutagenesis method using QuickChange Site-Directed Mutagenesis Kit (Stratagene). The method leading to an amino acid switch at position 433 from threonine to arginine included many steps, as described below.

i. Two complimentary primers of 27 bases in length containing the desired mutation nearly in the middle of the primer and having a minimum GC content of 42% and terminal bases in one or more C or G bases were prepared using Beckman Oligo 1000M DNA Synthesizer.

ii. Isolation of Human recombinant FMO3 DNA-A single colony from LB-ampicillin plate was inoculated into 2 ml LB medium containing ampicillin (50µg/ml) and was grown overnight at 37°C. The miniculture was diluted 1:100 into 200 ml of LP-Amp media and grown overnight at 37°C, 250 rpm. Cells were harvested by centrifugation at 2,500 x g for 10 min and plasmid DNA was isolated by QIAGEN plasmid purification protocol. To determine the yield, DNA concentration was measured at 260 and 280 nm in a UV spectrophotometer followed by analysis on an agarose gel.

iii. Restriction analysis of native and mutant recombinant FMO3 - Human FMO3 (native and mutant) was identified by restriction with enzyme EcoRI at 37°C, and then restriction fragments were electrophoresed in 1% agarose gels using TBE (Tris-borate-EDTA) Buffer.

iv. Mutagenesis - A series of sample reactions using various concentrations of ds FMO3 DNA template ranging from 5 to 50 ng were used. Each reaction contained 125 ng of oligonucleotide primers #1 and #2 and 10 mM dNTP mix (2.5 mM each NTP) in a final volume of 50 µl. Two synthetic primers containing the desired mutation, each complementary to the opposite strands of FMO, were extended during temperature cycling after adding 1 µl of Pfu DNA polymerase (2.5U/ml) using a Pelkin Elmer

GeneAmp PCR System 2400. Following temperature cycling, each amplification reaction was treated with 1 μ l of the Dpn I restriction enzyme (10U/ml) at 37°C for 1 hour to digest the parental nonmutated dsDNA template and to select for mutation-containing synthesized DNA. Circular, nicked dsDNA was transformed into Epicurian Coli XL 1-Blue supercompetent cells. Transformed bacterial cells were plated on LB-Amp and incubated at 37°C overnight. Bacterial colonies were placed in LB-Amp broth and incubated overnight with shaking at 250 rpm at 37°C. DNA isolated from overnight cultures by miniprep DNA isolation procedure was restricted and also sequenced as described before.

Assay of flavin-containing monooxygenase activity. For the measurement of the FMO activity and studying the effects of imipramine and chlorpromazine, substrate, methimazole was used. Methimazole S-oxidase activity was measured by the spectrophotometric method of Dixit and Roche (21) with 25-50 μ g of recombinant microsomal FMO3 protein. The reaction medium for methimazole activity measurements contained 100 mM Tris, pH 8.4, 0.06 mM DTNB, 0.02 mM DTT and 0.1 mM NADPH in final volume of 1 ml. Reaction was started with addition of methimazole. Kinetics of methimazole metabolism were determined from the results obtained by the addition of increasing amounts of methimazole (2-1000 μ M final concentrations) to the sample cuvette. In studying the effects to these drugs on methimazole activity, metabolism of imipramine and chlorpromazine was assessed. Methimazole metabolism with these drugs was studied using a methimazole concentration between 1-2000 μ M. The effect of the addition of imipramine (50-

750 μ M), chlorpromazine (1-200 μ M) on FMO3-mediated metabolism of methimazole was determined. The methimazole activity was measured initially in the absence of these drugs using recombinant FMO3 and this activity was considered as 100%.

Results

Production and expression of human FMO3 mutant. The nucleotide and amino acid sequences of FMO isoforms; human FMO3, rabbit FMO1, pig FMO1, rabbit FMO2 and rabbit FMO3 collected from GenBank were compared using GCG (Genetic Computer Group) programs. The amino acid region between 420 and 440 in the sequence was shown in Figure 1 and this region was found to be very conserved except a few changes among the FMO isoforms. Human FMO3 recombinant mutant was produced by changing the polar hydroxyl-containing amino acid threonine (position 433) to positively charged arginine, and this mutant was called hu FMO3 arg 433. The change of Thr to Arg was accomplished by point mutation on the triplet coding threonine (T), ACA (TGT), to the triplet coding arginine, AGA (TCT) using site-directed mutagenesis technique as described in 'Methods'. Both native and mutant human FMO3 were expressed in *E. coli*. The particulate fractions (microsomal fraction) from *E. coli* were used in methimazole assay for estimation of expression of FMO3 protein. The human FMO3 mutant expressed was found to be metabolically active.

Characterizations of native and mutant human FMO3: Metabolism of methimazole by human FMO3. The

Figure 1. Comparison of amino acid sequences of flavin monooxygenase isozymes (FMOs). Figure shows the region of 420-440. FMO sequences were obtained from Genbank and aligned using GCG programs.

420		433																				
R	K	W	E	G	K	.	.	S	E	T	I	Q	T	D	Y	I	V	Y	M	D	Human FMO3	
H	N	G	F	G	L	C	Y	C	K	A	L	Q	A	D	Y	I	T	Y	I	D	Rabbit FMO1	
P	S	G	F	G	L	C	Y	C	K	A	L	Q	S	D	Y	I	A	Y	I	D	Pig FMO1	
I	A	L	F	G	E	S	L	S	Q	K	L	Q	N	T	N	Y	I	D	Y	L	D	Rabbit FMO2
T	K	L	K	W	F	G	K	S	E	T	I	Q	T	D	Y	I	N	Y	M	D	Rabbit FMO3	

Human FMO3 433 Threonine (T) -----> Arginine (R)

ACA -----> AGA

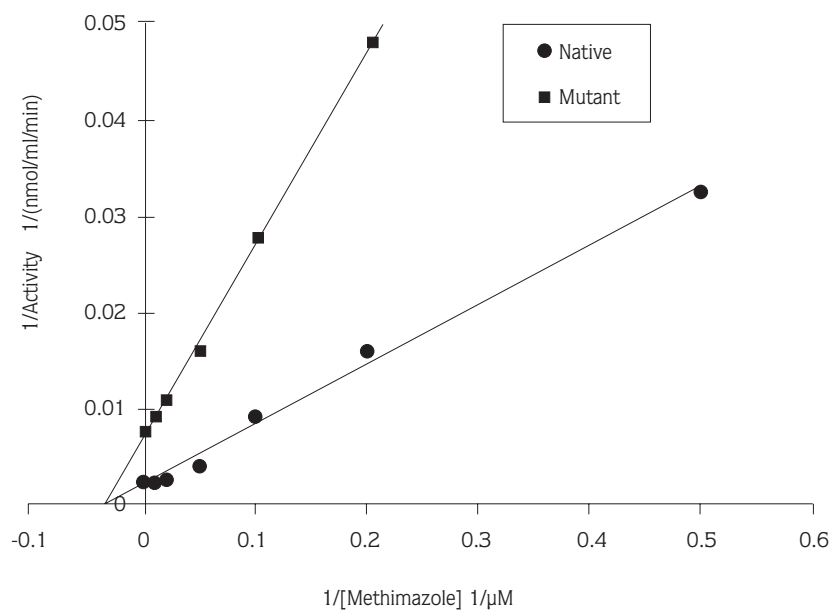


Figure 2. Metabolism of methimazole catalyzed by human liver FMO3 (A) or mutant FMO3 (B). Lineweaver-Burke plots are shown for the relationship between the rate of methimazole S-oxidation (1/nmol product/ml/min) and the concentration of methimazole (1/μM).

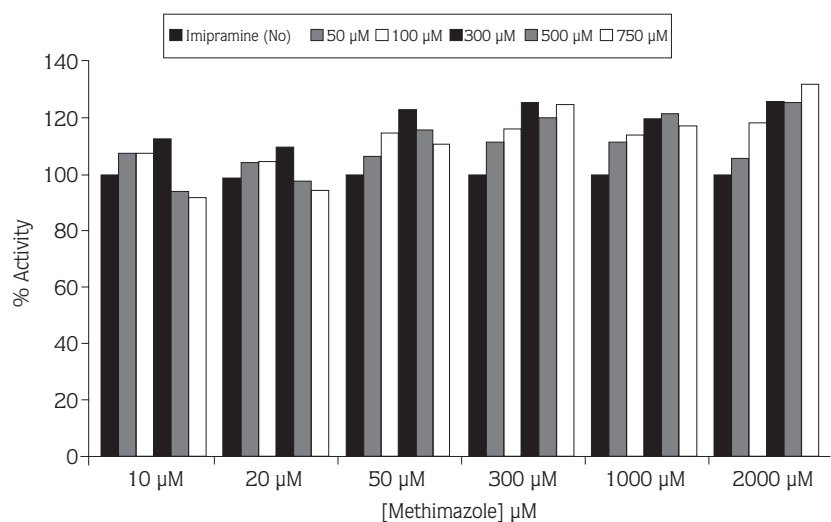


Figure 3. Effect of imipramine on the metabolism of methimazole catalyzed by human liver FMO3. The effects of five concentrations (50, 100, 300, 500 and 750 μM) of imipramine on the metabolism of methimazole (10, 20, 50, 300, 1000 and 2000 μM) are shown as a percent control activity (no imipramine).

functional properties of native and mutant human liver FMO3 were examined with methimazole as the substrate. Both native and mutant enzymes catalyzed the S-oxidation of methimazole to the same extent and showed a relationship between substrate concentration and rate of reaction. The kinetic data were expressed in the linearized form and the kinetic constants of native and mutant FMO3 for methimazole were calculated from the double reciprocal plots, as shown in Figure 2. The apparent Km values of native and mutant FMO3 for the methimazole reaction were found to be 30μM and 28.5

μM, respectively. The Vmax values of native and mutant FMO3 were calculated as 500 and 150nmol methimazole oxidized/min/ml, respectively. Differences in activities and therefore Vmax values reflect the variable levels of expression of native and mutant enzymes.

The effect of imipramine and chlorpromazine on the metabolism of methimazole catalyzed by native human FMO3. Expressed FMO3 was characterized by monitoring the methimazole metabolism in the presence of imipramine and chlorpromazine. Imipramine activation of methimazole metabolism was found to increase as the

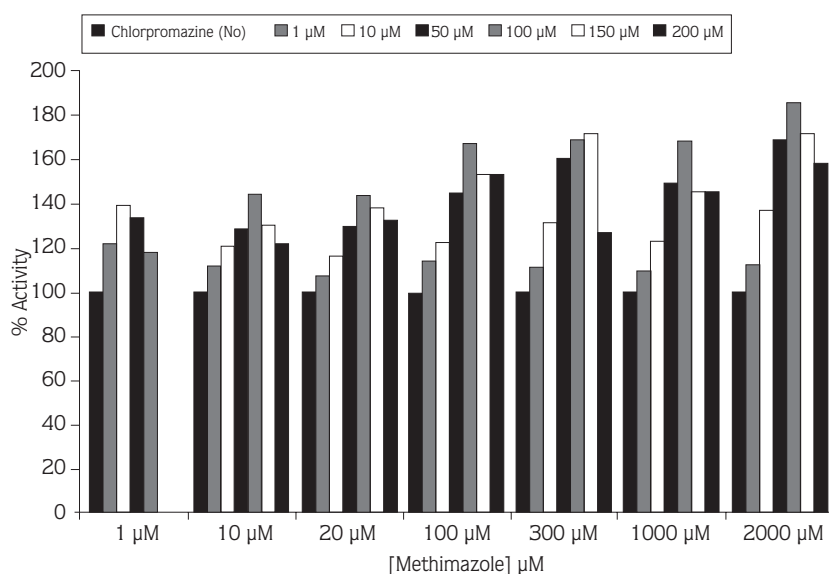


Figure 4. Effect of chlorpromazine on the metabolism of methimazole catalyzed by human liver FMO3. The effects of six concentrations (1, 10, 50, 100, 150 and 200 μM) of chlorpromazine on the metabolism of methimazole (1, 10, 20, 100, 300, 1000 and 2000 μM) are shown as a percent control activity (no chlorpromazine).

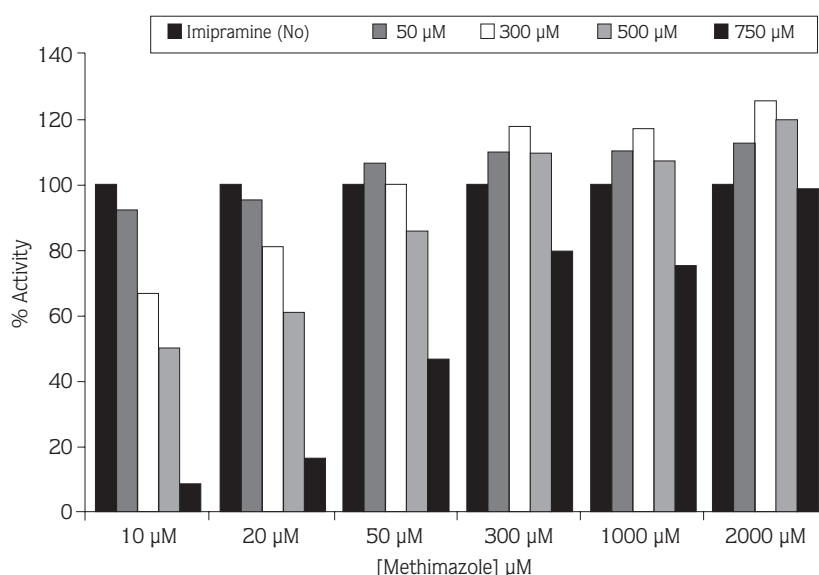


Figure 5. Effect of imipramine on the metabolism of methimazole catalyzed by mutant FMO3. The effects of four concentrations (50, 300, 500 and 750 μM) of imipramine on the metabolism of methimazole (10, 20, 50, 300, 1000 and 2000 μM) are shown as a percent control activity (no imipramine).

methimazole concentration increased from 10 μM to 2000 μM (Figure 3). Also, as the imipramine concentration increased from 50 μM to 750 μM , activation of methimazole reaction was found to be increased in general. However, 750 μM imipramine caused 5-10% inhibition of methimazole metabolism at 10 μM and 20 μM substrate concentrations. The maximum imipramine activation of about 30% was observed using a methimazole concentration of 2000 μM and an imipramine concentration of 750 μM . Chlorpromazine effect on methimazole activity of native human FMO3 was studied using 7 methimazole (1-2000 μM) and chlorpromazine (1-200 μM) concentrations. As shown in Figure 4, chlorpromazine caused a higher

activation of FMO3 catalyzed methimazole metabolism than imipramine. Activation was found to increase with increasing methimazole concentration. The greatest activation was found to be 85% when methimazole and chlorpromazine concentrations were 2000 μM and 100 μM , respectively. The inhibition of methimazole reaction beyond 100 μM chlorpromazine observed in all substrate concentrations was found to be due to the solubility of chlorpromazine and the turbidity of the reaction mixture.

Modulation of mutant human FMO3 catalyzed methimazole metabolism by imipramine and chlorpromazine. The imipramine effect on 433 arginine mutant catalyzed methimazole reaction differed from

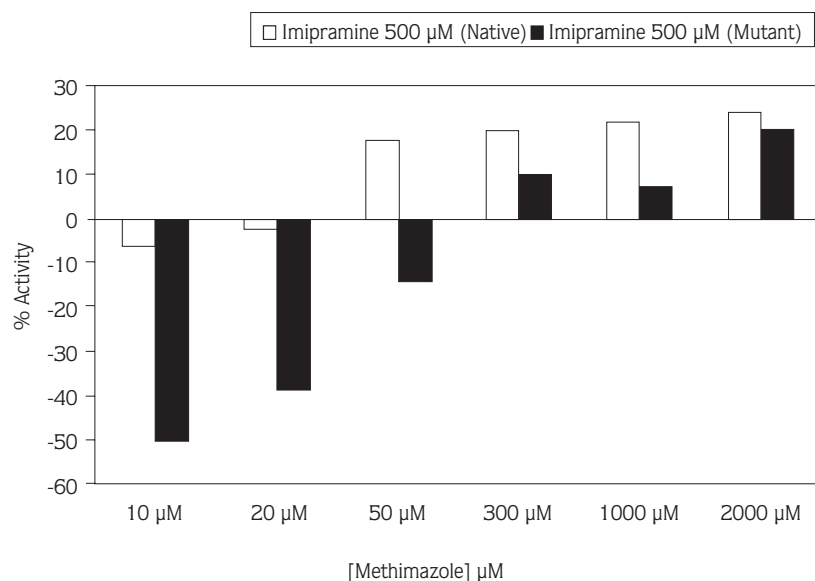


Figure 6. A comparison of 500 μM imipramine effect on methimazole activity of native and mutant human FMO3.

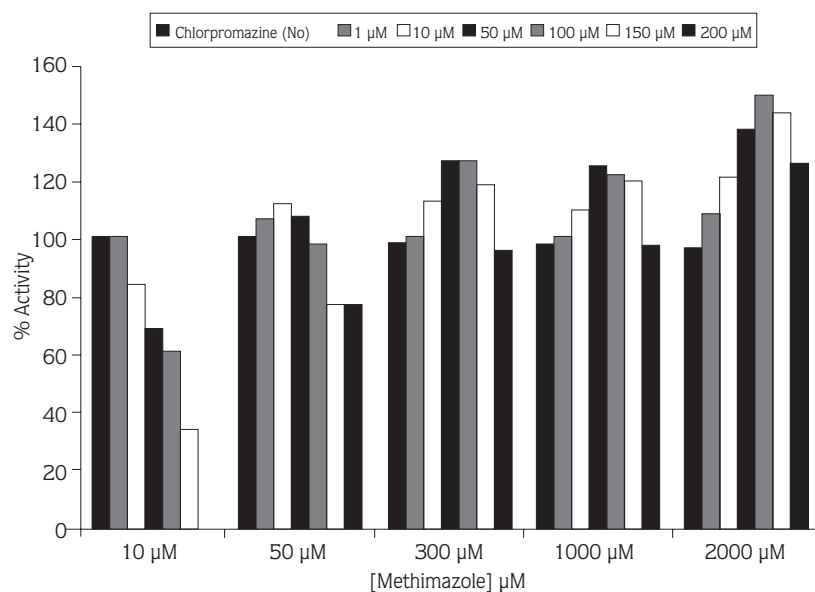


Figure 7. Effect of chlorpromazine on the metabolism of methimazole catalyzed by mutant FMO3. The effects of six concentrations (1, 10, 50, 100, 150 and 200 μM) of chlorpromazine on the metabolism of methimazole (10, 50, 300, 1000 and 2000 μM) are shown as a percent control activity (no chlorpromazine).

those observed with native FMO3 (Figure 5). The methimazole activity of mutant FMO3 was stimulated (maximally 25% when the methimazole concentration was 2000 μM) to the same extent of native enzyme up to an imipramine concentration of 300 μM. In contrast to results obtained with native FMO3, mutant enzyme methimazole activity was found to be decreased beyond the imipramine concentration of 300 μM. 750 μM imipramine caused a complete (93%) inhibition of the methimazole activity of the Mutant when the substrate concentration was 10 μM. The same concentration of imipramine caused 26% inhibition of activity when the

methimazole concentration was increased to 1000 μM. Imipramine neither caused inhibition nor activation when the methimazole concentration was 2 mM, so as the methimazole concentration was increased, imipramine inhibition beyond the 300 μM was decreased. A comparison of 500 μM imipramine effect on native and mutant human FMO3 catalyzed methimazole reaction is shown in Figure 6. At this imipramine concentration, the methimazole activity of native FMO3 was increased, whereas the activity of mutant enzyme decreased (or did not change significantly). These results showed that the extent of activation or inhibition of methimazole activity

by imipramine depends upon the concentration of methimazole and the structure of FMO3. In either case, activation or inhibition, a direct relationship was observed between the magnitude of effect and imipramine concentration. The effect of chlorpromazine on methimazole activity catalyzed by mutant FMO3 was found to be similar to the native enzyme. However, the magnitude of activation of methimazole activity of mutant FMO3 by chlorpromazine was found to be lower than that of native FMO3 (Figure 7). The maximum activation was observed with 100 μ M chlorpromazine. In contrast to native enzyme, chlorpromazine caused inhibition of activity when the methimazole concentration ranged between 10-20 μ M.

Discussion

It has been known for some time that the activation or inhibition of FMO-catalyzed reactions is dependent upon both the FMO isoform and the effector molecule. The FMO family consists of at least five isozymes, all of which have been identified in humans and rabbits (22). The primary structures of five FMO isoforms from the rabbit are 52-57% identical. Orthologs of the rabbit FMO isoforms present in other mammals, including humans, are 86-88% identical in primary structure to the rabbit forms (1). Preliminary work by Philpot and his associates (23) obtained by blot analysis of genomic DNA suggests that five genes present in the rabbit are also present in humans, guinea pigs, hamsters, rats, and mice. The involvement of FMO-mediated reactions in human drug metabolism has been associated with a number of substrates, including dimethylaniline (24), tertiary amines including imipramine (25) and chlorpromazine (3) and tamoxifen (4). On the basis of many studies (11-14) it has been suggested that FMO3 is the major isozyme responsible for FMO-mediated drug metabolism in the adult human liver.

The results of the present study show that in addition to FMO isoform and effector molecule, the manner and extent of modulation can also be a function of a third factor, substrate concentration. The methimazole activity of native and mutant human FMO3 increased as the

chlorpromazine concentration and substrate (methimazole) concentration increased. Imipramine affected the methimazole activity of native and mutant human FMO3 differently. Imipramine enhanced the methimazole activity up to a concentration of 300 μ M. Beyond this concentration, it caused inhibition of mutant methimazole activity. Therefore, imipramine and chlorpromazine are concentration dependent activator or inhibitor of human liver FMO3. With either inhibition or activation, the extent of the effect showed a similar dependency on imipramine and chlorpromazine concentration. This study along with many others, shows that the more interesting characteristics of the FMOs are those associated with modulation of catalytic activity. Two distinct types of activation have been described: first, increases in metabolism caused by the addition of modulators to the incubation; second, substrate selfoxidation of dimethylaniline by pig FMO1 can be enhanced by a number of short chain primary amines, including n-octylamine, that are not substrates for the enzyme. With other substrates, such as dimethylaniline, no selfactivation was apparent. In contrast, n-octylamine, which is a substrate for FMO2 (26), does not activate this isoform, whereas metabolism catalyzed by FMO2, but not by FMO1, can be enhanced by tricyclic antidepressants (25), compounds that have been reported to be substrates for FMO1 but not for FMO2. These results and other are consistent with the conclusion reached by Ziegler (1) that the FMO contains distinct catalytic and regulatory sites.

Conclusion

This study shows that the amine acid threonine at position 433 of human FMO3 is important for the modulation of methimazole activity by imipramine. The modulation of activity depends on the concentration of the modulator compound and substrate concentration.

I greatly acknowledge TÜBİTAK for supporting this work through a NATO B2 scholarship (Orhan Adalı) for 6 months.

References

1. Ziegler, D.M., Recent studies on the structure and function of multisubstrate flavin-containing monooxygenases. *Ann. Rev. Pharmacol Toxicol.*, 33. 179-199 (1993).
2. Lemoine, A., Johan, M., and Cresteil, T., Evidence for the presence of distinct flavin-containing monooxygenases in human tissues. *Arch. Biochem. Biophys.*, 276. 336-342 (1990).

3. Coccla, P.F and Westerfeld, W. W., The metabolism of chlorpromazine by liver microsomal enzyme systems. *J. Pharmacol. Exp. Therap.*, 157, 446-458. (1967).
4. Mani, C., Hodgson, E., and Kupfer, D., Metabolism of the antimammary cancer agent tamoxifen II. Flavin-containing monooxygenase-mediated N-oxidation. *Drug Metab. Dispo.*, 21, 657-661. (1993).
5. Lawton, M. P. and Philpot, R. M., Functional characterization of flavin-containing monooxygenase 1B1 expressed in *Saccharomyces cerevisiae* and *Escherichia coli* and analysis of proposed FAD-and membrane-binding domains. *J. Bio. Chem.*, 268, 5728-5734. (1993).
6. Duescher, R. J., Lewton, M.P. Philpot, R. M., and Elfarra, A.A., Flavin-containing monooxygenase (FMO)-dependent metabolism of methionine and evidence for FMO3 being the major FMO involved in methionine sulfoxidation in rabbit liver and kidney microsomes. *J. Biol. Chem.*, 269, 17525-17530. (1994).
7. Al-Waiz, M., Ayesh, R., Mitchell, S.C., Idle, J.R., and Smith, R.L., Trimethylaminuria (Fish-odour-syndroma): an inborn error of oxidative metabolism. *Lancet*, 1, 634-635. (1987).
8. Al-Waiz, M., Ayesh, R., Mitchell, S.C., Idle, J.R., and Smith, R.L., A genetic polymorphism of the N-oxidation of trimethylamine in humans. *Clin. Pharmacol. Ther.*, 42, 588-594. (1987).
9. Tynes, R.E. and Philpot, R.M., Tissue- and species- dependent expression of multiple forms of mammalian microsomal flavin-containing monooxygenase. *Mol. Pharmacol.*, 31, 569-575. (1987).
10. Ziegler, D.M., Flavin-containing monooxygenases: Catalytic mechanism and substrate specificity. *Drug Metab. Rev.* 19, 1-32. (1988).
11. Philips, I.R., Dolphin, P., Clair, P., Hadley, M.R., Hutt, A.J., McCombie, R.R., Smith, R.L. and Shephard, E.A., The molecular biology of the flavin-containing monooxygenases of man. *Chemico-Bio. Interact.*, 96, 17-32. (1995).
12. Lomri, N., Gu, Q., and Cashman, J.R., Molecular cloning of the flavin-containing monooxygenase (Form II) from adult human liver. *Proc. Natl. Acad. Sci. USA*, 92, 9910 (1995).
13. Cashman, J.R., Park, S.B., Yang, Z-C Washington, C.B., Gomex, D.Y., Giacomini, K.M., and Brett, C.M., Chemical, enzymatic, and human enantioselective S-oxidation of cimetidine. *Drug Metab. Dispo.*, 21, 587-597. (1993).
14. Overby, L.H., Carver, G.C., and Philpot, R.M., Quantitative analyses of human flavin-containing monooxygenase isoforms 3 and 5 in hepatic microsomal and recombinant preparations. *Arch. Biochem. Biophys.*, In Press.
15. Burnett, V.B., Lawton, M.P., and Philpot, R.M., Cloning, sequencing, and characterization of flavin-containing monooxygenases FMO3 and FMO4 from rabbit. *J. Biol. Chem.*, 269, 14314-14322. (1994).
16. Sadeque, A.J.M., Thummel, K.E., and Rettie, A.E., Purification of macaque liver flavin-containing monooxygenase: A form of the enzyme related immunochemically to an isozyme expressed selectively in adult human liver. *Biochim. Biophys. Acta.* 1162, 127-124. (1993).
17. Ozols, J., Isolation on structure of a third form of liver microsomal flavin monooxygenase. *Biochemistry*, 33, 3751-3757. (1994).
18. Krause, R.J., Ripp, S.L., Sausen, P.J., Overby, L., Philpot, R.M., and Elfarra, A.A., Characterization of the methionine S-oxidase activity of rat liver and kidney microsomes: Immunochemical and kinetic evidence for FMO3 being the major catalyst. *Arch. Biochem. Biophys.*, 333, 109-116. (1996).
19. Feinberg, A.P., and Vogelstein, B., A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.*, 132, 6-13. (1983).
20. Sanger, F., Nicklen, S., and Coulson, A.R., DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci.*, 74, 5463-5467. (1977).
21. Dixit, A. and Roche, T.E., Spectrophotometric assay of the flavin-containing monooxygenase and changes in its activity in female mouse liver with nutritional and diurnal conditions. *Arch. Biochem. Biophys.*, 223, 50-63. (1984).
22. Lawton, M.P., Cashman, J.R., Cresteil, T., Dolphin, C.T., Elfarra, A.A., Hines, R.N., Hodgson, E., Kimura, T., Ozols, J., Philips, I.R., Philpot, R.M., Poulsen, L.L., Rettie, A.E., Sheppard, E.A., Williams, D.E., and Ziegler, D.M., A nomenclature for the mammalian flavin-containing monooxygenase gene family based on amino acid sequence identities. *Arch. Biochem. Biophys.*, 308, 254-527. (1994).
23. Lawton, M.R. and Philpot, R.M., Emergence of the flavin-containing monooxygenase gene family. *Rev. Biochem. Toxicol.* (E. Hodgson, J.R. Bend, R.M. Philpot eds) 11. Toxicol Communications, Inc. Raleigh, NC. (1995), pp. 1-27.
24. McManus, M.E., Stupans I., Burgess, W., Koenig, J.A., de al Hall, P., and Birkett, D.J., Flavin-containing monooxygenase activity in human liver microsomes. *Drug Metab. Dispo.*, 15, 256-261. (1986).
25. Lemoine, A., Johann, M., and Cresteil, T., Evidence for the presence of distinct flavin-containing monooxygenases in human tissues. *Arch. Biochem. Biophys.*, 276, 336-342. (1990).
26. Poulsen, L.L., Taylor, K., Williams, D.E., Masters, B.S.S., and Ziegler, D.M., Substrate specificity of the rabbit lung flavin-containing monooxygenase for amines: Oxidation products of primary alkylamines. *Mol. Pharmacol.*, 30, 680-685. (1986).