FUNGAL BIOTRANSFORMATION STUDIES OF ZOLMITRIPTAN – A SUBSTRATE FOR CYP1A2

B.Sujitha, J.Sandhya Rani, P.Rajeswary, S.Varalaxmi, M.Vidyavathi*
Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, A.P
Email: vidyasur@rediffmail.com

Abstract— Human beings during their lives take a wide almost infinite variety of drugs into their bodies which are necessary for their well-being and these drugs undergo metabolism.Metabolism or biotransformation is defined as the structural or chemical alteration of a drug by enzymatic systems. Hence understanding of drug metabolism plays an important role in the development of new drug entities. Drug metabolism studies have relied on the use of model systems to produce the expected human metabolites of drugs. A microbial model is one of the *in vitro* model which constitute an alternative to the use of animal model and other *in vitro* models. The fungal biotransformation of a substrate of CYP1A2 [Zolmitriptan] was performed using six fungi in the present study. The screening studies were performed by selected fungi using their respective broth media. Fermentation was carried out for each organism by maintaining two controls and one sample to find the ability of fungi to biotransform the selected drug using HPLC method. Among all these organisms *Cunninghamella elegans* had shown an extra peak at 6.5min in HPLC when compared with its controls indicated the formation of its metabolite. The metabolite thus formed was identified, isolated and structure was confirmed by mass spectroscopy and NMR. Based upon the results obtained it was concluded that Zolmitriptan was biotransformed to its microbial metabolite i.e the sulphate conjugate of hydroxy indole acetic acid derivative of zolmitriptan by desmethylation, hydroxylation and sulphation of Zolmitriptan by *Cunninghamella elegans*. Further metabolism inhibition and induction studies were performed for the confirmation of enzyme CYP1A2 involved in biotransformation of Zolmitriptan by *Cunninghamella elegans*. There was no change in % of metabolite formed in presence of both inducer and inhibitor used. It indicated that *Cunninghamella elegans* converting the zolmitriptan to its metabolite not like by human CYP1A2 enzyme. Hence, the present study revealed that *Cunninghamella elegans* can be used as a suitable microbial model to study the biotransformation of drugs by different reactions to a new metabolite.

Index Terms— Zolmitriptan, biotransformation, fungi, *Cunninghamella elegans*, microbial metabolite.

———————————— ◆ ————————————

IJSER

1 INTRODUCTION

Human beings during their lives take a wide almost infinite variety of drugs into their bodies which are necessary for their well-being and these drugs undergo metabolism [1].Metabolism or biotransformation is defined as the structural or chemical alteration of a drug by enzymatic systems. Hence understanding of drug metabolism plays an important role in the development of new drug entities [2]. The knowledge of drug metabolism is also important for studying metabolism induction or inhibition of several drugs [3]. The approval and usage of drug in human subjects require extensive studies to establish its safety and efficacy. Predicting the metabolism and toxicity of a drug in humans can use resources that include *in vivo* animal models, *in vitro* systems, and genomics and proteomics methods. Drug metabolism studies have relied on the use of model systems to produce the expected human metabolites of drugs. Generally, whole animal systems are used, especially small laboratory animal models [e.g., rat, dog, cat, guinea pig, and rabbit] or *in vitro* using tissue culture, microsomal preparations, or perfused organ systems. There are large number of drawbacks associated with the use of the above models. A microbial model is one of the *in vitro* model which constitute an alternative to the use of animal model and other *in vitro* models. In addition, several observations on fungi reported to possess monooxygenase enzyme systems which were similar to the mammalian hepatic monooxygenases. The use of microorganisms for simulating the mammalian metabolism of many molecules of pharmacological importance is well documented [4,5,6,7,8,9,10]. The present study was intended to develop a microbial model for the drug Zolmitriptan which is a substrate for CYP1A2.

2 PROCEDURE FOR PAPER SUBMISSION

2.1 Fungi

The fungi used in present study were *Aspergillus flavus*[MTCC 1783], *Aspergillus ochraceus* [NCIM 1140], *Aspergillus terreus* [NCIM 657], *Cunninghamella echinulata* [NCIM 691], *Cunninghamella elegans* [NCIM 689], *Gliocladium roseum* [NCIM 1064]. These cultures were maintained on potato dextrose agar slants at 4 ºC and subcultured for every 6 months for maintaining their viability [11].

2.2 Chemicals

Zolmitriptan was obtained from Mylan laboratories, Hyderbad, India. Acetonitrile, Methanol and Water used for analysis were HPLC grade. Media components were obtained from Qualigens, and SD fine chemicals, Mumbai, India.

2.3 Media Composition

Potato dextrose broth [Potato chips, 20 gm / 100 ml [steamed for 30min], dextrose 2 gm, yeast extract 10 mg, distilled water up to 100 ml] was used for the fungi selected in the present study.

ISSN 2229-5518

2.4 Microbial Fermentations

The fermentation was carried by 50ml potato dextrose broth medium in 250ml Erlenmeyer flasks incubated for about 48 hrs, operated at 120 rpm at 37°C on the orbital shaker. The fermentation was performed by [Blank I] drug control which had only drug, [Blank II] culture control inoculated with respective fungus and sample flask had drug along with fungus to study Zolmitriptan biotransformation for each fungus.

2.5 Inhibition and Induction studies

Induction and inhibiton studies were performed by CYP1A2 inducer [carbamazepine] and inhibitor [ciprofloxacin] [12,13,14] for confirmation of enzyme involved in microbial metabolism. Blank I was used as substrate control, composed of sterile medium to which substrate was added and incubated without culture. Blank II was used as a culture control, composed of fermentation blanks in which the microorganisms were inoculated but without substrate addition. Blank III was used as inhibitor or inducer control, composed of sterile medium to which inhibitor or inducer was added and incubated without culture. Blank IV substrate and inhibitor or inducer control. Sample composed of sterile medium to which substrate, inhibitor or inducer was added and microorganism was inoculated. Thus, 5 flasks were prepared in inhibition or induction studies and incubated for 72 hrs on orbital shaker incubator [15].

2.6 Extraction Procedure

2.6 Analytical techniques

High Performance Liquid Chromatography:

The analysis of Zolmitriptan and its metabolite was performed by High Performance Liquid Chromatography [HPLC] method. The HPLC system [Waters, USA] consisted of Waters 515 solvent delivery module and Waters 2489 UV-visible spectrophotometric detector. The mobile phase comprised of Sodium acetate [buffer]: Methanol: Acetonitrile [70:20:10v/v] with a flow rate of 1ml / min. The column used was C-18 stainless steel column of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5 µ diameter, 100 Å pore diameter – II 5C-18 rs – 100a, 5 µm, 4.6 x 250 mm]. The elute was monitored at 225 nm, sensitivity was set at 0.001 a.u.f.s. [17].

Mass spectroscopy:

The metabolite was collected from HPLC elute after finding the metabolite peak in HPLC analysis and dried for further analysis using mass spectroscopy operating in the electron spray ionization (ESI) mode. Model was Agilent 1100 Series mass spectrometer operating in the electron spray ionization (ESI) mode. Detector used was ion trap detector, operated at positive mode, range: 50-700, spray voltage: 3.5 kV, capillary temperature: 325°C, nebulizer gas pressure: 210 psi.

PNMR Spectroscopy:

The metabolite collected from HPLC elute was dried and its structure was further confirmed by PNMR spectroscopy by using BRUKER AVANCE 400 MHz [IICT, Hyderabad]. Deuterated Methanol was employed as solvent to analyze PNMR spectra of Zolmitriptan and its metabolite.

3 RESULTS

In present study the biotransformation of Zolmitriptan was performed using six fungal organisms. The HPLC analysis of Zolmitriptan and its metabolite in different culture extracts was conducted and its results are represented in Table.1. The peaks at retention time of 2.7 min represented solvent peak and peaks at 3.1min., 4.0min., 5.1min, represented various culture contents. The peak at retention time of 9.0 min. indicated Zolmitriptan based on retention time of pure Zolmitriptan. Interestingly the sample of *Cunninghamella elegans* shown an extra metabolite peak at 6.5min. Compared to its controls as shown in Figure.1. The structure of metabolite was confirmed by Mass spectroscopy and PNMR techniques.

Table 1. HPLC data for Zolmitriptan and its metabolite from different microbial culture extracts.

Name of the organism	Retention time (min)			
	Blank I	Blank II	(Control) pure Zolmitriptan	Sample
Aspergillus flavus MTC1783	2.7	2.7	-	2.7
	3.1	3.1	-	3.1
	-	4.0	-	4.0
	-	5.2	-	5.2
	9		9	9
Aspergillus terreus NCIM657	2.7	2.7	-	2.7
	3.1	3.2	-	3.1
	-	4.3	-	4.3
	-	5.2	-	5.2
	9		9	9
Cunnighamella elegans NCIM 689	2.7	2.7	-	2.7
	3.1	3.1	-	3.1
	-	4.0	-	4.0
	-	5.2	-	5.2
				6.5*
	9		9	9
Aspergillus Ochraceus NCIM 1140	2.9	2.9	-	2.9
	3.3	3.3	-	3.3
	-	4.2	-	4.2
	-	5.4	-	5.4
	9		9	9
Gliocladiumroseum NCIM 1064	2.8	2.8	-	2.8
	3.1	3.2	-	3.1
	-	4.0	-	4.0
	-	5.2	-	5.2

IJSER

	9		9	9
Cunninghamella echinulata MTCC 4279	2.7	2.8	-	2.7
	3.1	3.2	-	3.1
	-	4.0	-	4.0
	-	5.2	-	5.2
	9	-	9	9

***- Metabolite**

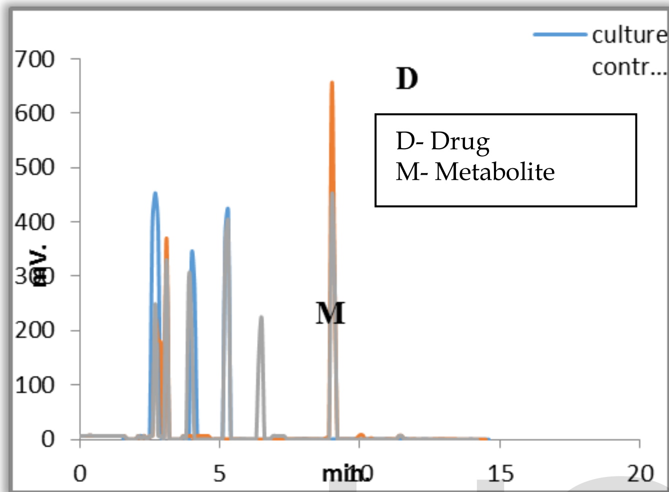

Figure.1: HPLC chromatogram of Zolmitriptan from culture extracts of *Cunninghamella elegans*

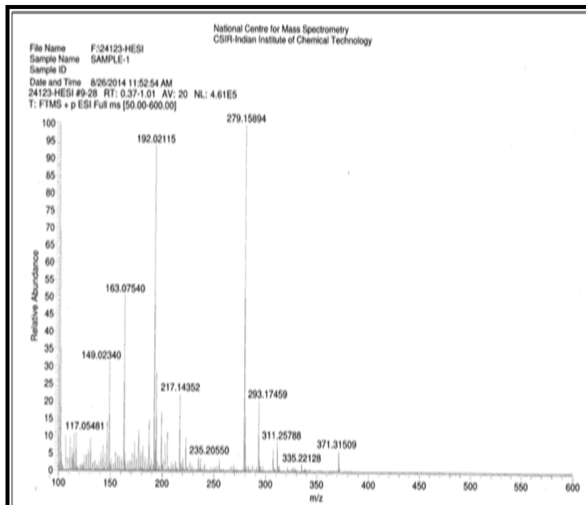

Figure.2 Mass spectrum of Zolmitriptan metabolite.

Mass spectrum of pure drug shown a molecular ion peak at m/z 288 [m+1] which corresponds to molecular weight of pure drug Zolmitriptan. The mass spectrum of metabolite shown a molecular ion peak at m/z 371 [M+1] as shown in figure.2 with fragmentation ions peaks at m/z 295[m+1], 279[m+1], & 235[m+1

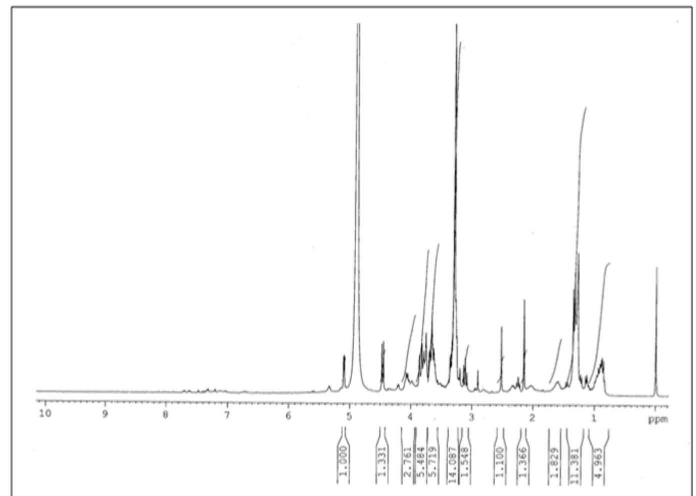

Figure.3 PNMR spectrum of Zolmitriptan metabolite

PNMR spectra of Zolmitriptan metabolite is given in Figure.3 which has shown a singlet at δ2.8 which represents the hydroxyl group proton attached to benzene ring so it confirms the hydroxyl indoleacetic acid derivative of Zolmitriptan.

4 DISCUSSIONS

Six fungi were screened in the present study for microbial metabolism of Zolmitriptan. Screening studies for metabolism of zolmitriptan was performed using fermentation technique and HPLC method. The sample of *Cunninghamella elegans* shown an extra metabolite peak at retention time of 6.5min. Compared to its controls in HPLC analysis as given in Table.1 and Figure.1. The extra peak represented the formation of zolmitriptan metabolite by *Cunninghamella elegans*. Other microbes shown identical peaks in sample and controls which indicated that, the organisms screened, could not metabolize the drug. It was further analysed by LCMS and PNMR studies to confirm the structure of metabolite formed.

The mass spectrum of pure Zolmitriptan exhibited a molecular ion peak at m/z 288 [M+1] which corresponds to the molecular weight of Zolmitriptan. The mass spectrum of metabolite of Zolmitriptan shown a molecular ion peak at m/z 371[M+1], further fragmentation ions of metabolite are observed at m/z 295[m+1], 279[m+1], 235[m+1] as shown in Figure.2, which represented as sulphate conjugate of hydroxy indole acetic acid derivative of zolmitriptan.

Further, to confirm the structure of sulphate conjugate of hydroxy indole acetic acid metabolite of Zolmitriptan, the PNMR spectra of Zolmitriptan and its metabolite were compared. The presence of doublelet peaks at δ1.3, δ1.4, δ1.5 indicate **methylene group protons**, peaks at the range of δ2 to δ4 **methylene group protons**, peaks at the range of δ4.5 to δ4.9 are due to aromatic protons, peak at δ5 is due to amine group protons of Zolmitriptan structure. The PNMR spectrum of Zomitriptan metabolite showed a singlet at δ2.8 which is due to hydroxyl

IJSER

groups protons attached to the benzene ring as shown in Figure 3 which confirms the hydroxylation of Zolmitriptan.

Hence based on the obtained results, the present investigation found N-desmethylation of Zolmitriptan and further formation indole acetic acid derivative is like in mammals, but further hydroxylation of indole acetic acid derivative by phase I reaction to the hydroxy indole acetic acid and sulphation by phase II reaction to form the sulphate conjugate of hydroxy indole acetic acid metabolite of Zolmitriptan was formed by *Cunninghamella elegans.* Thus, it indicates that the fungus *Cunninghamella elegans* has the ability to metabolize the Zolmitriptan by hydroxylation of indole acetic acid metabolite and further by sulphation. Then induction and inhibition studies were performed to determine the type of enzyme involved in microbial metabolism of Zolmitriptan. No change in % of metabolite formed was observed in the metabolism induction and inhibition studies by CYP1A2 inducer [Carbamazepine] and inhibitor [Ciprofloxacin]. It indicates that CYP1A2 is not involved in the microbial metabolism of Zolmitriptan as there was no change in percentage metabolite formed in presence of inducer and inhibitor during in induction and inhibition studies.

5 CONCLUSION

On the basis of above results and discussion, the present study revealed that *Cunninghamella elegans* can biotransform the Zolmitriptan to sulphate conjugate of hydroxy indole acetic acid derivative of zolmitriptan by desmethylation, hydroxylation and sulphation. CYP enzyme involved in mammalian metabolism of Zolmitriptan is CYP1A2. But in present study, the percentage of metabolite of Zolmitriptan formed by *Cunninghamella elegans* was unchanged in presence of inhibitor and inducer of CYP1A2. Hence, it can be concluded that *Cunninghamella elegans* has the ability to biotransform the Zolmitriptan by not CYP1A2 like enzyme but may be by other microbial enzyme to sulphate conjugate of hydroxy indole acetic acid derivative of Zolmitriptan. So, *Cunninghamella elegans* can be used as a microbial model for biotransformation of the drugs to form new metabolites by different enzyme which can be used for further pharmacological and toxicological studies.

6 ACKNOWLEDGMENT

The authors are very thankful to UGC & Sri Padmavathi Mahila University for providing all the facilities to carryout this work.

7 REFERENCES

[1] E. A. Abourashed, A. M. Clark, and C. D. Hufford. Microbial models of mammalian metabolism of Xenobiotics: an updated review. *Curr. Med. Chem.*; 6: 359-374, 1999.

[2] S. Asha, and M. Vidyavathi, Cunninghamella – A microbial model for drug metabolism studies. A review. *Biotechnol. Adv.*; 27: 16-2, 2009.

[3] R. Azerad. Microbial models for drug metabolism. *Adv. Biochem. Eng. Biotechnol.* 63: 169-218, 1999.

[4] B. Kilic, T. Ozden, S. Toptan, and S. Ozilhan. Simultaneous LC–MS–MS determination Of Zolmitriptan and its active metabolite N-desmethylzolmitriptan in human plasma, 66, S129–S133, 2007.

[5] R. Beukers, A.F. Marx, M.H.J Zuidweg. Microbial conversion as a tool in the preparation of drugs. In: Ariens EJ, Editor. Drug Design, Vol.3, New York: Academic Press, 1, 1972.

[6] A.M. Clark, C.D. Hufford. Use of microorganisms for the study of drug metabolism – an update. *Med. Res. Rev*; 11: 473-501, 1991.

[7] A.M. Clark, J.D. Mc Chesney, C.D. Hufford. The use of microorganisms for the study of drug metabolism. *Med. Res. Rev*; 5: 231-253, 1985.

[8] M.S. Faber, A. Jetter, and U. Fuhr. Assessment of CYP1A2 activity in clinical practice: why, how, and when? *Basic Clin Pharmacol Toxicol.*; 97:125-134, 2005.

[9]M.J. Karjalainen, P.J. Neuvonen, and J.T. Backman , *In vitro* inhibition of CYP1A2 by model inhibitors, anti-inflammatory analgesics and female sex steroids: predictability of in vivo interactions. *Basic Clin Pharmacol Toxicol.*, 103(2):157-65, 2008.

[10] L.A. Kroon. Drug interactions with smoking. *Am J Health Syst Pharm*; 64:1917-1921, 2007.

[11] Kummarigunta Kavitha, Maravajhala Vidyavathi, Sepuri Asha, and T.V.L. Hima Bindu, Microbial Metabolism and Inhibition Studies of Phenobarbital. *Tropical Journal of Pharmaceutical Research.* 2012; 11 (1): 62-68.

[12] M. Vidyavathi, B. Sarika, and S. Varalakshmi, Fungal biotransformation of fenofibrate. *Indian journal of pharmaceutical education and research*, 48(1): 56-60, 2014.

[13] R.V. Smith, and J.P. Rosazza. Microbial models of mammalian metabolism, aromatic hydroxylation. *Arch. Biochem. Biophys*; 161: 551-8, 1974.

[14] R.V. Smith, and J.P. Rosazza Microbial models of mammalian metabolism. *J. Pharm. Sci*; 64: 1737-58, 1975.

[15] R.V. Smith, and J.P. Rosazza. Microbial models of mammalian metabolism. *J. Nat. Prod*; 46: 79-91, 1983.

[16] Zunjian Zhang, Fengguo Xu, Yuan Tian, Wei Li, and Guoguang Mao. Quantification of Zolmitriptan in plasma by high-performance liquid chromatography–electrospray ionization mass spectrometry. *Journal of Chromatography B*, 813, 227–233, 2004.

IJSER