

Fungal Biotransformation of Fenofibrate

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ABSTRACT

Screening studies were performed using eight fungal organisms for their potential to biotransform the drug fenofibrate to its active metabolite fenofibric acid. Among all fungi screened, only *Aspergillus terreus* shown an extra peak at 2.64 m compared to its controls indicating formation of metabolite. The metabolite thus formed was identified, isolated and structure was confirmed by HPLC, LCMS, IR and PNMR. *Aspergillus terreus* biotransformed the fenofibrate to its metabolite fenofibric acid. The biotransformation of fenofibrate by *Aspergillus terreus* was similar to metabolism of drug in mammals. Hence microorganisms can be used as a suitable tool for prediction of mammalian drug metabolism and to produce active metabolite of fenofibrate to use as new drug entity.

Keywords: Biotransformation, fungi, fenofibrate fenofibric acid, *Aspergillus terreus*.

INTRODUCTION

The ability of humans to metabolize and clear drugs is a natural process.¹ Drug metabolism leads to chemical alteration of the drug that is, conversion of drug to more polar and hydrophilic metabolites which are easily excreted from the body.² Most of the compounds are metabolized by Cytochrome P450 (CYPs) in human liver by a biotransformation interaction with biological systems.³ Hence understanding of drug metabolism plays an important role in the development of new drug entities.⁴ The approval and usage of drug in human subjects require extensive studies to establish its safety and efficacy. The important factor in the evaluation of safety and efficacy of any drug is the knowledge about drug metabolism.^{5,6,7}

Drug metabolism studies have relied on the use of whole animal systems especially small laboratory animal models or *in vitro* using tissue culture, microsomal preparations or perfused organ systems. Microbial model is one of the *in vitro*

model which constitute an alternative to the use of animal model and other *in vitro* models.⁸ Therefore it is a complementary tool in investigation of drug metabolism.^{9,10} Microorganisms such as fungi, bacteria and yeast have been used as *in vitro* models for prediction of mammalian drug metabolism.¹¹⁻¹³ In present study fenofibrate was selected for the microbial biotransformation studies as it is metabolized to an active metabolite in human. Fenofibrate is used in the treatment of type II a, type II b, type III, type IV and type V hyper lipoproteinaemias.¹⁴ Fenofibrate is metabolized in several stages. First the carboxyl ester moiety is cleaved by hydrolysis resulting in fenofibric acid the main pharmacologically active compound. Fenofibric acid in turn undergoes carbonyl reduction resulting in a pharmacologically active metabolite referred to as reduced fenofibric acid.¹⁵ Thus the present study aimed at development of microbial model for production of fenofibric acid an active metabolite of fenofibrate easily and cheaply.

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MATERIALS AND METHODS

Fungi

Aspergillus terreus (NCIM 657), *Aspergillus flavus* (MTCC 1783), *Aspergillus ochraceus* (NCIM 1140), *Cunninghamella blakesleeana* (MTCC 3729), *Cunninghamella elegans* (NCIM 689), *Cunninghamella echinulata* (MTCC 4279), *Rhizopus stolonifer* (NCIM 880), *Gliocladium roseum* (NCIM 1064). These fungal cultures were obtained from National Chemical Laboratory (NCL), Pune and Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Chemicals

Fenofibrate (99.43%) was obtained from Therdose Private Limited, Hyderabad, India. All solvents used for analysis were HPLC grade. Deionized and glass distilled water was used for this study, culture media components were purchased from Qualigens, and SD fine chemicals, Mumbai, India.

Media for cultures

All the fungal cultures were maintained on the respective agar slants at 4°C and transferred for every 6 months to maintain viability. Potato dextrose broth (Potato chips, 20gm/100ml (steamed for 30min); dextrose 2gm; yeast extract 10mg; distilled water up to 100ml), for *Cunninghamella elegans*, *Cunninghamella echinulata*, *Aspergillus terreus*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Gliocladium roseum* and *Rhizopus stolonifer*; Oat meal flakes, 3gm/100ml (steamed for 30min) for *Cunninghamella blakesleeana*.

Microbial biotransformation

The fermentation was carried out in 250ml erlenmeyer flasks containing 50ml medium were incubated on the orbital shaker for 24 to 48h, operated at 120rpm at 37°C labelled as drug control, culture control and sample. For each biotransformation study two controls and one sample were used. The study included mainly the drug control to which drug solution was added and incubated without organism. Culture control which consisted of the medium inoculated with only loop full of respective fungus to study fenofibrate biotransformation during incubation period.

Extraction procedure

The incubated flasks were taken out from shaker incubator and heated on water bath at 50°C for 30 min and centrifuged at 3000rpm for 10min (R8C: Remi instruments, Mumbai, India). Thus obtained supernatant was collected in separate boiling tubes from each flask of each culture. The extraction of fenofibrate and its metabolite is conducted by treating

the supernatant with a mixture of dichloromethane and methanol. The organic layer was collected and was evaporated by air drying. The dried extract was reconstituted with mobile phase for HPLC analysis.

ANALYTICAL TECHNIQUES

High performance liquid chromatography

Fenofibrate and its metabolite in the extracted samples were estimated by High Performance Liquid Chromatography (HPLC) method.¹⁶ The HPLC system (Waters, USA) consisted of Waters 515 solvent delivery module and Waters 2489 UV-visible spectrophotometric detector. The mobile phase consisted of Acetonitrile: Water (70:30v/v) with a flow rate of 1ml/min. The column used was C-18 (stainless steel column of 25cm length and 4.6mm internal diameter packed with porous silica spheres of 5µ diameter, 100 Å pore diameter – II 5C-18 rs – 100a, 5µm, 4.6×250mm). The eluent was monitored at 286nm., sensitivity was set at 0.001 a.u.f.s.

Mass spectrometry

The sample of *Aspergillus terreus* culture showed a metabolite peak at retention time of 2.64min in HPLC compared to their controls. Hence metabolite was collected from HPLC elute and dried for further analysis by mass spectrometry operating in the electron spray ionization (ESI) mode. Model was Agilent 1100 Series LC-MS/MS. LC coupled to a 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: 3.5kV, capillary temperature: 325°C, nebulizer gas pressure: 210psi.

IR spectrometry

The metabolite collected from the culture extracts of *Aspergillus terreus* and pure fenofibrate were subjected to IR analysis using BRUKER ALPHA-E.

PNMR spectrometry

The metabolite collected from HPLC elute was dried and its structure was confirmed by PNMR spectrometry by using BRUKER AVANCE 400MHz (Hyderabad central university, Hyderabad). Deuterated chloroform was employed as solvent to analyze PNMR spectra of fenofibrate and its metabolite.

RESULTS

Eight fungi were screened in the present study for microbial metabolism of fenofibrate. The results of HPLC analysis of fenofibrate and its metabolite in different culture extracts are represented in Table No. 1.

HPLC chromatogram of sample of *Aspergillus terreus* shown an extra metabolite peak at retention time of 2.64min compared to its controls as given in Table No. 1 and Fig. 1. The extra peak represented the formation of fenofibrate metabolite by *Aspergillus terreus*. Other microbes shown identical peaks in sample and controls which indicated that the organisms screened could not metabolize the drug. To confirm the structure of metabolite formed it was analysed by LCMS, IR and PNMR studies. Fenofibrate in mammals is being converted to its active metabolite fenofibric acid by hydrolysis. The metabolite produced was collected from elution of HPLC to analyse by LCMS, IR and PNMR.

The mass spectrum of pure fenofibrate exhibited a molecular ion peak at m/z 361.1(M+1) and was supported by a fragment ion peaks at m/z 139(M+1) and m/z 233(M+1) as shown in the Fig. 2. The mass spectrum of metabolite of fenofibrate showed a molecular ion peak at m/z 319.7(M+1) as shown in Fig. 3 which represents as fenofibric acid formed by hydrolysis of fenofibrate as in mammalian metabolic pathway. The structure of metabolite is supported by its fragment ion peaks at m/z 89, 233.

IR spectra of fenofibrate and its metabolite were compared to confirm the structure of fenofibrate metabolite as fenofibric acid. The presence of O-H bond at 3300cm^{-1} and presence of carbonyl C-O peaks at 1701 and 1674cm^{-1} and absence of ester C-O peak at 1721cm^{-1} as in Fig. 4 confirmed the structure of metabolite as fenofibric acid.

Further to confirm the structure of fenofibric acid the PNMR spectra of fenofibrate and its metabolite were compared. The absence of doublet due to $-\text{CH}(\text{CH}_3)_2$ group, multiplet due to CH group and presence of singlet peak at δ 11–12 due to COOH group Fig. 5 in PNMR spectrum of metabolite confirmed the structure of metabolite as fenofibric acid.

The formation of fenofibric acid by hydrolysis using microbes is similar to human beings as reported by John Caldwell.¹⁶ Hence based on the obtained data the present investigation found the hydrolysis of fenofibrate by phase I reaction to its metabolite fenofibric acid by *Aspergillus terreus* is similar to mammals. Thus the fungus *Aspergillus terreus* has the ability to metabolize the fenofibrate by hydrolysis to fenofibric acid and it can be used as a model for production of active metabolites by hydrolysis.

CONCLUSION

Among all selected fungi *Aspergillus terreus* only has the ability to metabolize fenofibrate by hydrolysis to fenofibric acid similar to mammals. Thus this organism can be used as a complementary tool for prediction of

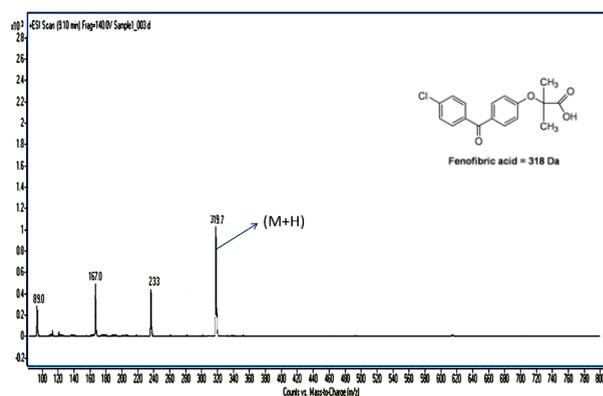


Figure 3: Mass Spectrum of Fenofibrate Metabolite Produced By *Aspergillus Terreus*.

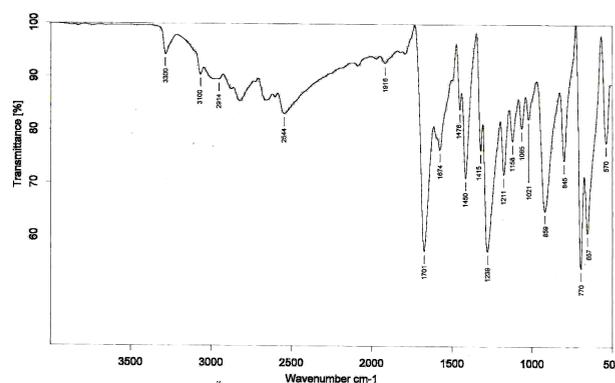


Figure 4: IR Spectrum of Fenofibrate Metabolite Produced By *Aspergillus Terreus*.

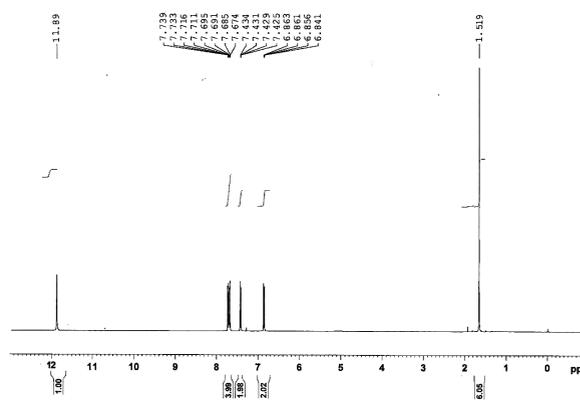


Figure 5: PNMR Spectrum of Fenofibrate Metabolite Produced by *Aspergillus Terreus*.

mammalian drug metabolism and production of active metabolite to develop new drug entities.

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