Studies on microbial transformation of albendazole by soil fungi

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Received 8 September 2008; revised 5 March 2009; accepted 3 May 2009

Soil enrichment technique was followed to isolate the fungi capable of performing biotransformation of albendazole. Among the 5 fungi isolated, Aspergillus fumigatus, A. niger and Penicillium chrysogenum could transform albendazole to one metabolite and Fusarium moniliforme could transform albendazole to two metabolites. The transformation was confirmed by HPLC. Based on LC-MS-MS analysis, the metabolites formed were predicted to be albendazole sulfoxide and albendazole sulfone. The results support that the soil enrichment is a promising technique for isolation of fungi with industrial applicability, viz. production of active metabolites from drugs.

Keywords: Albendazole sulfoxide, albendazole sulfone, biotransformation, fungi

Introduction

Microorganisms and their enzymes have proved to be versatile biocatalysts1 and are involved in the transformation of complex organic materials and some of them have the potential to metabolize or degrade the desired compound. Such microorganisms can be isolated by enrichment techniques. Biotransformation encompasses practically every type of chemical reaction possible and is extremely useful since some of these reactions can be carried out more economically. Unlike traditional chemical processes, which require extreme temperatures and pressures, microbial conversions take place under mild conditions and, in some instances, the products are formed stereoselectively. The interest in bioconversion is mainly because the product of the process is more useful or valuable than the precursor used. The transformation of organic compounds using biocatalysts, cell organelles or whole cells are important processes in organic synthesis and have been widely used in the production of steroids, antibiotics, vitamins and other high valuable products2. Use of biocatalyst also minimizes the problems of isomerization, racimisation, epimerization, and rearrangement that are common in chemical processes3.

Albendazole (ABZ) (methyl 5-propylthio-1H-benzimidazol-2-y carbamate) is a broad spectrum anthelmintic of the benzimidazole group. It is effective in the treatment of several parasitic diseases. ABZ is widely used in veterinary and human therapy4. In animals, after parenteral, oral or intraruminal administration, ABZ is rapidly oxidized to its sulfoxide5-7. Albendazole sulfoxide (ABZSO), known as ricobendazole, is responsible for the anthelmintic activity as well as for the potential embryotoxicity in some animal species8,9. ABZSO undergoes bioconversion to albendazole sulfone (ABZSO2), which is pharmacologically inactive10,11. The flavin containing monoxygenases (FMO) and cytochromes P450 (CYP, mainly CYP3A in rat) appear to mediate the conversion of ABZ to ABZSO, whereas the biotransformation of ABZSO to ABZSO2 is influenced by CYP only (CYP1A in rat)12-14. ABZSO has been established as an active metabolite8,9. Due to limited absorption and rapid metabolism, only high and prolonged doses are effective against systemic infections such as hydatid disease and neurocysticercosis15. To increase the bioavailability, an injectable formulation of ABZSO has been developed. After subcutaneous injection, ABZSO is widely distributed to various tissues, especially the gastrointestinal tract16 so that plasma concentrations closely reflect those in fluids and tissues where the target parasite may be located17.
In our previous work on biotransformation of albendazole by fungi\textsuperscript{18} we reported the production of ABZSO, ABZSO\textsubscript{2} and N-methylated metabolite of ABZSO. Present investigation is aimed to isolate an organism from soil that is capable of transforming albendazole to ABZSO, the active metabolite of albendazole.

**Materials and Methods**

**Microorganisms**

Soil enrichment technique\textsuperscript{19} was followed to isolate fungi which are capable of carrying out biotransformation. Rhizosphere soil samples from 10-15 cm depth, after removing 1-2 cm top soil, were collected from the Botanical Garden of Kakatiya University, and brought to the laboratory. One gram of sample was collected in the test tube containing 9.0 mL of sterilized distilled water and subjected to shaking. From this solution, serial dilutions were prepared to get a dilution of 10\textsuperscript{-4}. One mL of the dilute solution was taken aseptically into sterilized Petri plates of 90 mm diameter. Then 20 mL of sterile Asthana and Hawkers medium A (Glucose, 5 g; KNO\textsubscript{3}, 3.5 g; KH\textsubscript{2}PO\textsubscript{4}, 1.75 g; MgSO\textsubscript{4}, 0.75 g; agar agar, 15g; and distilled water, up to 1000 mL) containing 0.02% w/v of albendazole was poured with gentle rotational movement of Petri plates so as to ensure uniform spreading and mixing of the sample. Petri plates thus prepared were incubated in an inverted position at 27°C for 5 d. Strains which have the capacity to tolerate and metabolize albendazole were enriched and selected in the medium. The fungi thus developed in the Petri plates were isolated and identified with the help of standard monographs\textsuperscript{20-24} after recording morphological features. The cultures were preserved on potato dextrose agar (PDA) slants.

**Chemicals**

Albendazole was gifted by GlaxoSmithKline, Mumbai, India. Methanol and acetonitrile were of HPLC grade obtained from Ranbaxy, New Delhi, India. Peptone, yeast extract, glucose, potato dextrose agar, and all other chemicals of highest available purity were obtained from Himedia, Mumbai, India.

**Biotransformation**

Biotransformation was performed using a two-stage fermentation protocol. In the first stage, fermentation was initiated by inoculating a 250 mL culture flask containing 50 mL of liquid broth. The liquid broth used contained (per L) glucose (20 g), peptone (5 g), yeast extract (5 g), K\textsubscript{2}HPO\textsubscript{4} (5 g) and sodium chloride (5 g). The pH of the broth was adjusted to 6.0 with 0.1 N HCl. The prepared media was autoclaved and cooled to room temperature. The media was inoculated with a loopful of spores obtained from freshly grown (7 d) PDA slants. The flasks were incubated at 120 rev/min and 28°C for 48 h. Second stage cultures were initiated in the same medium using an inoculum of 1.0 mL portion from first stage culture per 20 mL of medium in a 100-mL culture flask. The second stage cultures were incubated for 24 h and the substrate albendazole in dimethyl formamide was added to give a final concentration of 100 mg/L. The flasks were incubated under similar conditions for 5 d. Culture controls consisted of a fermentation blank in which the microorganism was grown under identical conditions and no substrate was added. Substrate controls comprised of albendazole added to the sterile medium were incubated under similar conditions. The experiments were conducted in triplicate with each species. The cultures were extracted by liquid-liquid extraction using separating funnel with three volumes of ethyl acetate, the combined organic extracts were evaporated using a rotary vacuum evaporator and dried over a bed of sodium sulfate. The resultant residues were analysed by HPLC and LC-MS-MS for identification of metabolites.

**Analysis**

The transformation was identified by HPLC analysis according to the method described by Garcia \textit{et al}\textsuperscript{25} with a slight modification. The samples were analysed using an LC-10AT system (Shimadzu, Japan) by injecting 20 μL of sample into the syringe-loading sample injector (Model 7725i, Rheodyne, USA). The column used was Wakosil II, C18, 250 X 4.6 mm and 5 μm (SGE, Australia). The mobile phase consisted of a mixture of acetonitrile-water (pH adjusted to 3.0 with orthophosphoric acid) in the ratio of 15:85. The analysis was performed isocratically at a flow rate of 1 mL/min and the analytes were detected at 290 nm using a photodiode array detector (Model SPD M10Avp, Shimadzu, Japan). LC-MS-MS analysis was carried out using a Waters system, column XTerra C18, 25 X 0.46 cm, 5 μm and a mobile phase consisting of acetonitrile and water (pH adjusted to 3.0 with formic acid) in 15:85 ratio. The ESI detection was set to positive mode. A temperature of 300°C and a scan range of 50-500 were set for the analysis. The transformed compounds were identified from the masses of the fragmentation products obtained.
Results and Discussion

In order to isolate native fungi capable of transforming albendazole, soil enrichment technique was adopted. The fungi appeared on Petri plates were screened for their potential to transform albendazole and the results are presented in Table 1. Among different fungi screened, two species of Aspergillus, one Fusarium species and one Penicillium species were proved to be potential to transform albendazole, except a strain of Fusarium.

Fusarium moniliforme isolated by soil enrichment technique transformed albendazole into ABZSO (M₂) and ABZSO₂ (M₁). In support to our findings, Uzura et al.²⁶ performed stereoselective oxidation of alkyl benzenes by employing F. moniliforme isolated by soil enrichment technique. Whereas Aspergillus fumigatus, A. niger and Penicillium chrysogenum could transform albendazole to yield ABZSO (M₂). Similarly, pentachlorophenol transforming P. veronii was isolated by selective enrichment of soil samples.²⁷ ABZSO and ABZSO₂ could not be detected in the culture control and substrate control.

Albendazole metabolites formed were identified based on observation of new peaks in HPLC (Fig. 1) and characterized with the help of the mass values of fragmentation ions obtained in LC-MS-MS analysis (Fig. 2). Mass spectrometric analysis of the metabolite M₂ showed a molecular ion at m/z 281 (an increase of 16 units) indicating addition of single oxygen atom to albendazole, which results in formation of ABZSO (M₂). Another molecular ion was found at m/z 298 (an increase of 32 units) indicating addition of two oxygen atoms to albendazole, which results in the formation of ABZSO₂ (M₁). Data obtained by MS-MS analysis of M₂ showed fragment ions at m/z 240 (loss of 42 units) and 208 (further loss of 32 units). MS-MS fragment route of albendazole sulfoxide is shown in Fig. 3.

These analyses indicated that the metabolites were ABZSO (M₂, 20.78 min) and ABZSO₂

![Fig. 1—HPLC chromatograms of culture control and transformed compounds by F. moniliforme.](image1)

![Fig. 2—LC-MS-MS spectra of metabolites detected in albendazole fed culture broth of F. moniliforme.](image2)

![Fig. 3—MS-MS fragment route of albendazole sulfoxide obtained in culture broth of F. moniliforme.](image3)

<table>
<thead>
<tr>
<th>Culture</th>
<th>% Metabolites formed</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albendazole sulfone(M1)</td>
<td>Albendazole sulfoxide(M2)</td>
<td>Albendazole remaining</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>0.00</td>
<td>5.55</td>
<td>94.45</td>
</tr>
<tr>
<td>A. niger</td>
<td>0.00</td>
<td>7.33</td>
<td>92.66</td>
</tr>
<tr>
<td>F. moniliforme</td>
<td>4.22</td>
<td>10.51</td>
<td>85.26</td>
</tr>
<tr>
<td>Fusarium sp</td>
<td>0.00</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td>P. chrysogenum</td>
<td>0.00</td>
<td>5.50</td>
<td>94.50</td>
</tr>
</tbody>
</table>

Table 1—Biotransformation of albendazole by soil fungi

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²⁶ Uzura, et al
²⁷ Kowalski, et al
(M1, 11.56 min) where the substrate albendazole was eluted at 27.81 min. The predicted pathway of the metabolite formation is shown in Fig. 4. The metabolites were quantified, based on the peak areas obtained in HPLC analysis, taking the peak areas of drug and metabolites together as 100%.

Present studies on the transformation of albendazole revealed the possibilities of its giving rise to a variety of derivatives by employing fungi isolated by soil enrichment technique. Out of different fungi isolated, *F. moniliforme* could transform albendazole to ABZSO and ABZSO2. *F. moniliforme* was previously reported in biotransformation of various aromatic compounds28. Whereas *A. fumigatus, A. niger* and *P. chrysogenum* could transform albendazole to ABZSO only. Similar type of oxidation reactions were reported earlier29,31. In mammals, flavin containing monoxygenases (FMO) and cytochromes P450 (CYP, mainly CYP3A in rat) appear to mediate conversion of ABZ to ABZSO, whereas the biotransformation of ABZSO to ABZSO2 is influenced by CYP only (CYP1A in rat)12-14. Similar enzyme systems may be involved in the transformation of albendazole to ABZSO and ABZSO2 by the fungi employed in the present investigation.

In the present investigation, it is interesting to note that the transformation of albendazole was found to be oxidative in nature. In various mammals, albendazole undergoes similar metabolic pathways. These metabolic conversions included oxidation at sulfur alkyl and aromatic hydroxylation, methylation at both nitrogen and sulfur and carbamate hydrolysis32. Albendazole sulfoxide and albendazole sulfone were identified in plasma after oral administration in several species viz., rat, human, porcine, ovine, bovine, caprine and chicken12,14,17,33-36.

In our experiment to isolate and develop an efficient microbial conversion of albendazole to obtain ABZSO, *A. fumigatus, A. niger* and *P. chrysogenum* could transform albendazole to ABZSO, whereas *Fusarium moniliforme* could transform albendazole to ABZSO and ABZSO2 in significant quantities.

**Conclusion**

From the present investigation it is concluded that ABZSO, the active metabolite of albendazole with increased anthelmintic activity can be produced by employing fungi isolated by soil enrichment technique. However, further investigations are needed to optimize the conditions responsible for production of ABZSO in large quantities, viz., by exclusively directing the transformation towards ABZSO.

**References**


Fig. 4—Metabolic pathway of albendazole in *F. moniliforme*. 


24 Sutton B C, *The coelomycetes fungi imperfecti with pycnidia, acervuli and stromata* (Commonwealth Mycological Institute, Kew, UK) 1980.


