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Biotransformation of metoprolol by the fungus *Cunninghamella blakesleeana*

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Key words

metoprolol; biotransformation; *Cunninghamella blakesleeana*; liquid chromatography-tandem mass spectrometry

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Abstract

Aim: To investigate the biotransformation of metoprolol, a β_1-cardioselective adrenoceptor antagonist, by filamentous fungus, and to compare the parallels between microbial transformation and mammalian metabolism. **Methods**: Five strains of *Cunninghamella* (*C elegans* AS 3.156, *C elegans* AS 3.2028, *C echinulata* AS 3.2004, *C blakesleeana* AS 3.153 and AS 3.910) were screened for the ability to transform metoprolol. The metabolites of metoprolol produced by *C blakesleeana* AS 3.153 were separated and assayed by liquid chromatography-tandem mass spectrometry (LC/MS^n). The major metabolites were isolated by semipreparative HPLC and the structures were identified by a combination of LC/MS^n and nuclear magnetic resonance analysis. **Results**: Metoprolol was transformed to 7 metabolites; 2 were identified as new metabolites and 5 were known metabolites in mammals. **Conclusion**: The microbial transformation of metoprolol was similar to the metabolism in mammals. The fungi belonging to *Cunninghamella* species could be used as complementary models for predicting *in vivo* metabolism and producing quantities of metabolite references for drugs like metoprolol.

Introduction

Metoprolol, 1-isopropylamino-3-(4-[2-methoxyethyl]-phenoxy)-2-propanol, is a β1 selective aryloxypropanolamine used in treatment of cardiovascular disorders such as hypertension, arrhythmia, and heart failure. The drug is a lipophilic adrenoceptor antagonist (β-blocker) with a short half life (3–4 h)[1]. It is therapeutically used as a racemic mixture.

Metoprolol is mainly eliminated by the hepatic oxidative metabolism and it undergoes extensive first pass metabolism with about 95% of the dose being metabolized in humans. Metoprolol is metabolized to a large degree by cytochrome P-450 2D6 which is polymorphic in the human population. The appearance of major metabolites *O*-desmethyl-metoprolol, metoprolol acid, and hydroxymetoprolol varies depending on the oxidation phenotype[2–4].

The identification of metabolites from mammalian sources may be hindered by insufficient quantities of material. The concept of using microorganisms, particularly the filamentous belonging to the genus *Cunninghamella*, as models of mammalian metabolism has been well documented[5–7]. The advantages of a microbial system as an *in vitro* model for drug metabolism include its low cost, ease of handling, scale-up capacity, and potential to reduce the use of mammals. A microbial system also provides enough putative metabolites under milder conditions than those required by chemical systems. Thus, fermentation is used to scale up the synthesis of metabolites for structural confirmation by nuclear magnetic resonance (NMR)[8].

Although the metabolism of metoprolol in mammals has previously been studied, to our knowledge, no report has been published on the potential of filamentous fungi to metabolize metoprolol. Cultures of 5 strains of *Cunninghamella* were chosen for this investigation, because such fungi have the ability to metabolize drugs, including verapamil[9], pantoprazole[10], and indomethacin[11], in a similar manner to mammals. The main focus of the current study was to investigate the metabolic fate of metoprolol in cultures of the 5 strains of *Cunninghamella* and demonstrate parallels of the metabolism of metoprolol in mammalian and in microbial systems.

Materials and methods

Materials Metoprolol (purity 99.0%) was provided by Xinhua Pharmaceutical Co (Changzhou, China); the purity was checked by HPLC analysis. Methanol of HPLC grade was purchased from Concord Technology (Tianjin, China). Peptone and the yeast extract were biochemical reagents. All other chemicals were of analytical grade.

Microorganisms *C elegans* AS 3.156, *C elegans* AS 3.2028, *C echinulata* AS 3.2004, and *C blakesleeana* AS 3.153 and AS 3.910, were provided by the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). Stock cultures were maintained on potato dextrose agar (Aoboxing, Beijing, China) slants at 4 °C and transferred every 6 months to maintain viability. The first-stage microbial biotransformation was carried out in a medium consisting of 20 g dextrose, 5 g peptone, 5 g yeast extract, 5 g NaCl, 5 g K_2HPO_4, and 1000 mL distilled water. The second-stage biotransformation was carried out in a wheat-bran medium containing 1% wheat-bran in broth. The pH of the medium was adjusted to 6.5 with 6.0 mol/L HCl, and then the medium was sterilized in Erlenmeyer flasks (Pierce, Rockford, IL, USA) at 115 °C and 18 psi for 30 min and cooled before incubation.

Biotransformation procedures The microbial metabolism was facilitated by incubating the cultures with shaking on a rotary shaker at 28 °C. For each of the 5 strains of *Cunninghamella*, the first-stage preculturing was initiated by inoculating a 250-mL Erlenmeyer flask containing 50 mL broth with a loop of spores obtained from a freshly growing agar slant. After incubation for 24 h, a 1.0 mL portion from the first-stage culture was used to inoculate a second-stage 100-mL flask containing 20 mL of broth. The second-stage culture was incubated for 24 h before metoprolol was added to a final concentration of 1.0 g/L. After 120 h of additional incubation, the culture was centrifuged at 1500×*g* for 20 min, and the supernatant was decanted and kept at -20 °C until analysis.

The preparative-scale biotransformation of metoprolol by *C blakesleeana* AS 3.153 followed the same procedure as the screening experiments, except for the increase of the broth volume. Two first-stage flasks were prepared as described earlier, and 4 second-stage flasks each containing 100 mL of broth were incubated with 2.5 mL of the first-stage culture. Metoprolol was added to yield a final concentration of 1.0 g/L. The culture was then incubated for an additional 120 h.

Two kinds of controls were conducted simultaneously with the biotransformation procedure. Culture controls consisting of a fermentation blank, in which the microorganisms were grown under the same conditions without metoprolol, were operated to eliminate the interference possibly brought by the microorganism itself or residues of the fermentation cultures. The substrate controls were prepared by adding metoprolol to sterile medium and incubated without the microorganism to determine whether metoprolol could chemically decompose or spontaneously transform under fermentation conditions.

Extraction of metabolites and liquid chromatography-tandem mass spectrometry (LC/MS^n) assay A 0.5 mL aliquot of each sample was filtered through a membrane (0.45 μm pore size). The filtrate was applied to a preconditioned Orgchem C_{18} cartridge (Orgchem, Troy, NY, USA). The cartridge was washed with 1.5 mL water, and the metabolites were eluted with 2.0 mL methanol. A 20 mL aliquot of the eluate was directly injected into the LC/MS^n system for analysis.

The LC/MS^n analysis was performed on a Thermo Finnigan LCQ ion trap mass spectrometer (San Jose, CA, USA) equipped with an atmospheric-pressure ionization interface. The instrument was operated in positive electrospray ionization (ESI) mode. The spray voltage was set at 4.5 kV. The capillary temperature was maintained at 200 °C and the voltage was fixed at 13 V. The HPLC fluid was nebulized by using N_2 as both the sheath gas at a flow rate of 0.75 L/min and the auxiliary gas at a flow rate of 0.15 L/min. A full-scan mass spectrum was operated to obtain the protonated molecules $(M+H)^+$ of each possible metabolite. Multi-stage mass spectra (MS/MS or MS^3) were produced by collision-induced dissociation of the selected precursor ions with helium present in the ion trap, and the relative collision energy was set at 30%–35%. Data were collected and analyzed with Xcalibur software (version 1.2, Thermo Finnigan, NJ, USA). Liquid chromatography was carried out with a Shimadzu LC-10AD solvent delivery system (Kyoto, Japan). The samples were separated on a Diamonsil C_{18} column (200 mm×4.6 mm inner diameter, 5 μm, Dikma, Beijing, China) preceded by a SecurityGuard C_{18} guard column (4 mm×3.0 mm inner diameter, 5 μm, Phenomenex, Torrance, CA, USA). The mobile phase consisted of methanol-water-formic acid (35:65:0.2, *v:v:v*) at a flow rate of 0.5 mL/min.

Isolation and identification of major metabolites To isolate the major metabolites of metoprolol in sufficient quantities for structure elucidation, the biotransformation was carried out on a semipreparative scale. After biotransformation, the fluid was centrifuged at 1500×*g* for 20 min. The supernatant was condensed using a Rikakikai Eyela Fdu1100 freeze dryer (Tokyo, Japan). The residue was reconstituted by 10 mL methanol-water (1:1, *v:v*) and directly

deposited onto a 5 cm diameter column packed with Sephadex G10 gel (Zhonghuida Scientific Instrument Co, Dalian, Liaoning, China). The sample was washed exhaustively with methanol and the eluent was collected every 50 mL. The solvent of each fraction was analyzed directly by LC-MS^n. Fractions containing possible metabolites were evaporated to dryness under vacuum at 40 °C using a RE-52A rotary evaporator (Yarong Biology Instrument, Shanghai, China). In order to obtain sufficient quantities of metabolites for structural identification, the sample was dissolved in a small amount of water and then injected repeatedly onto a semipreparative HPLC system (Shimadzu, Kyoto, Japan) consisting of 2 LC-6AD solvent delivery units (Shimadzu, Kyoto, Japan), a DGU-14A degasser unit (Shimadzu, Kyoto, Japan), a SCL-10A VP system controller (Shimadzu, Kyoto, Japan), a SPD-10A VP UV-Vis detector (Shimadzu, Kyoto, Japan), a FRC-10A (Shimadzu, Kyoto, Japan) fraction collector, and a CLASS-VP LC workstation (Shimadzu, Kyoto, Japan). Separation was accomplished using a mobile phase consisting of methanol-water (30:70, *v*:*v*) at 8.0 mL/min on a Shim-Pack PRC-ODS column (250 mm×20 mm inner diameter, Shimadzu, Kyoto, Japan) preceded by a GPRC-ODS pre-column (8 mm×1.5 mm inner diameter, Shimadzu, Kyoto, Japan). The UV detector was set at 223 nm. The major peaks with similar retention time were pooled, evaporated to dryness, and stored at 4 °C before the structural analysis.

The purified metabolites were dissolved in D_2O for the NMR analysis. The NMR measurements were carried out at 600 MHz on a Bruker ARX 600 NMR spectrometer (Bruker, Faellanden, Switzerland). Chemical shifts were reported as parts per million relative to tetramethylsilane as the internal standard.

Results

Screening of cultures Five strains of *Cunninghamella* were screened and all could transform metoprolol to some extent. The percentages of transformation were 10.1%

Table 1. Investigation of the biotransformation conditions, including the type of medium, pH, and concentration of the substrate for metoprolol transformed by *C blakesleeana* AS 3.153. *n*=3 in triplicate.

Number	Factors			Yield (%)
	A (Substrate, g/L)	B (Medium)	C (Initial pH)	X
1	1 (0.342)	1 (common culture)	1 (4.5)	28.25
2	1 (0.342)	2 (wheat-bran)	2 (6.5)	33.85
3	1 (0.342)	3 (soybean powder)	3 (8.5)	37.35
4	2 (1.00)	1 (common culture)	2 (6.5)	34.29
5	2 (1.00)	2 (wheat-bran)	3 (8.5)	42.71
6	2 (1.00)	3 (soybean powder)	1 (4.5)	47.18
7	3 (1.37)	1 (common culture)	3 (8.5)	35.92
8	3 (1.37)	2 (wheat-bran)	1 (4.5)	49.21
9	3 (1.37)	3 (soybean powder)	2 (6.5)	29.93
		Results analysis		
	X	X	X	
K_{1j}	99.45	98.46	124.64	
K_{2j}	124.18	125.77	98.07	
K_{3j}	115.05	114.46	115.98	
K'_{1j}	33.15	32.82	41.55	
K'_{2j}	41.39	41.92	32.69	
K'_{3j}	38.35	38.15	38.66	
R_j	8.24	9.10	8.86	
Optimum	A_2	B_2	C_1	

X, amount of *O*-desmethylmetoprolol, metoprolol acid, and hydroxymetoprolol compared to the total amount of metabolites and remained substrate.

(*C elegans* AS 3.156), 12.6% (*C elegans* AS 3.2028), 82.7% (*C blakesleeana* AS 3.153), 15.3% (*C blakesleeana* AS 3.910), and 3.69% (*C echinulata* AS 3.2004). Since *C blakesleeana* AS 3.153 transformed the highest proportion of metoprolol, it was selected for further investigation. The type of medium, pH, and concentration of the substrate were also investigated using an L_9 (3^4) orthogonal table (Table 1). According to Table 1, the first choice for biotransformation should be at pH 4.5 in a wheat-bran medium with a substrate concentration of 1.0 g/L ($A_2B_2C_1$). However, the factor of pH was found to have no influence on the final yield. Finally biotransformation was carried out at pH 6.5 in a wheat-bran medium with a final substrate concentration of 1.0 g/L.

Identification of metoprolol metabolites The LC/MS^n chromatograms of the culture control showed no spontaneous formation of possible metabolites of metoprolol under the same conditions. The substrate control contained only metoprolol. As shown in Figure 1, with the exception of metoprolol, 7 possible metabolites were detected in the *C blakesleeana* AS 3.153 cultures, compared with the control cultures.

Following the transformation of metoprolol by *C blakesleeana* AS 3.153, 2 major metabolites (M1 and M2) were isolated by semipreparative HPLC, and their structures were identified by a combination analysis of LC/MS^n and NMR spectra. The other metabolites were tentatively identified based on their retention time and MS^n information (Table 2).

Parent drug The identification of metoprolol (M0) was confirmed by comparison of the retention time and mass spectra (Figure 2) with the authentic reference. The compound eluting at 10.5 min was identified as metoprolol. In order to elucidate the structures of metabolites through the mass spectra, the cleavage pathways of metoprolol were stud-

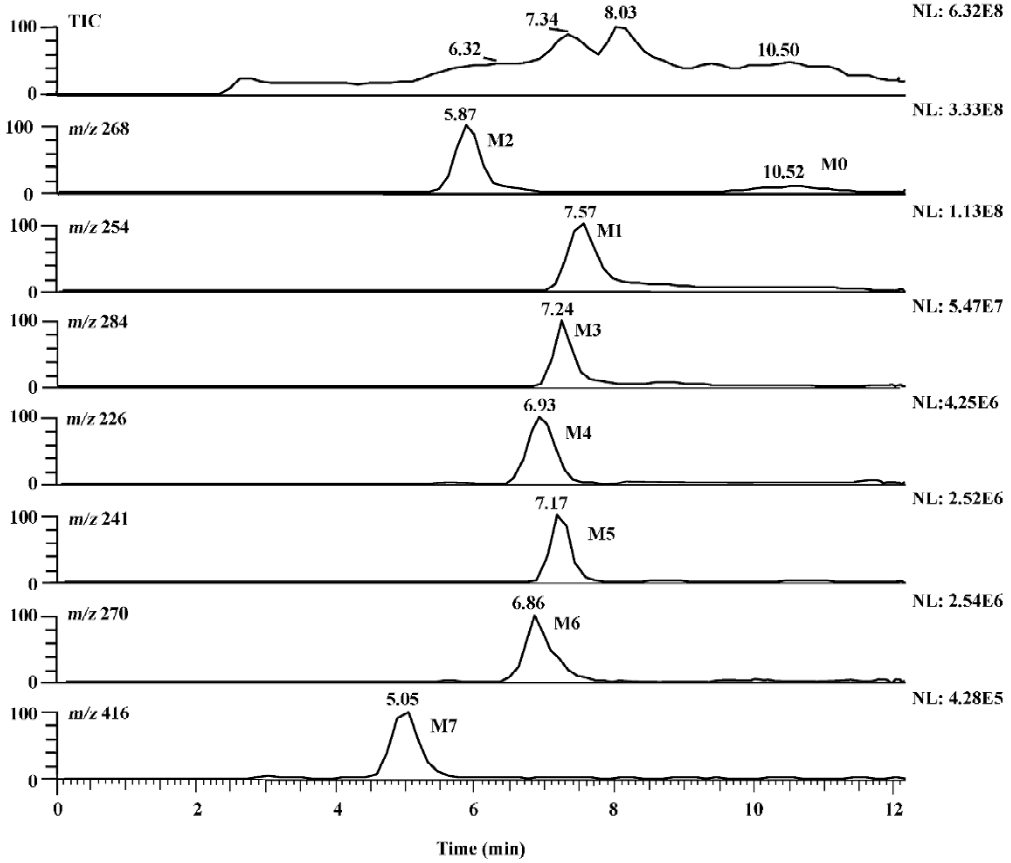

Figure 1. Total ion current (TIC) and full MS/MS scan chromatograms of metoprolol and its metabolites in the sample of *C blakesleeana* AS 3.153 incubated in wheat-bran broth with 1.0 g/L metoprolol for 120 h. NL, normalized *y*-axis normalization mode in which the instrument automatically sets the vertical scale equal to the height of the largest peak; M0, metoprolol; M1, *O*-desmethylmetoprolol; M2, metoprolol acid; M3, α-hydroxymetoprolol; M4, *N*-dealkylmetoprolol; M5, deaminated metoprolol; M6, hydroxyl-*O*-desmethylmetoprolol; M7, glucoside conjugate of *O*-desmethylmetoprolol.

Table 2. Chromatographic and mass spectral data for metoprolol and its metabolites produced by *C blakesleeana* AS 3.153.

Compound	Retention time (min)	MS $(M+H)^+$	MS/MS (*m/z* [relative abundance])[a]
M0	10.52	268	250(20), 226(28), 218(42), 191(100), 176(30), 116(56), 98(25)
M1	7.57	254	236(35), 212(45), 177(100), 116(40), 98(22)
M2	5.87	268	250(22), 226(45), 191(100), 116(28), 98(22)
M3	7.24	284	252(100), 224(35), 207(58), 175(20), 116(90)
M4	6.93	226	208(42), 191(100), 179(25), 74(30)
M5	7.17	241	223(100), 197(56), 165(29)
M6	6.86	270	252(35), 228(70), 193(100), 116(24)
M7	5.05	416	254

[a] Only the abundant ions (>10%) are shown.

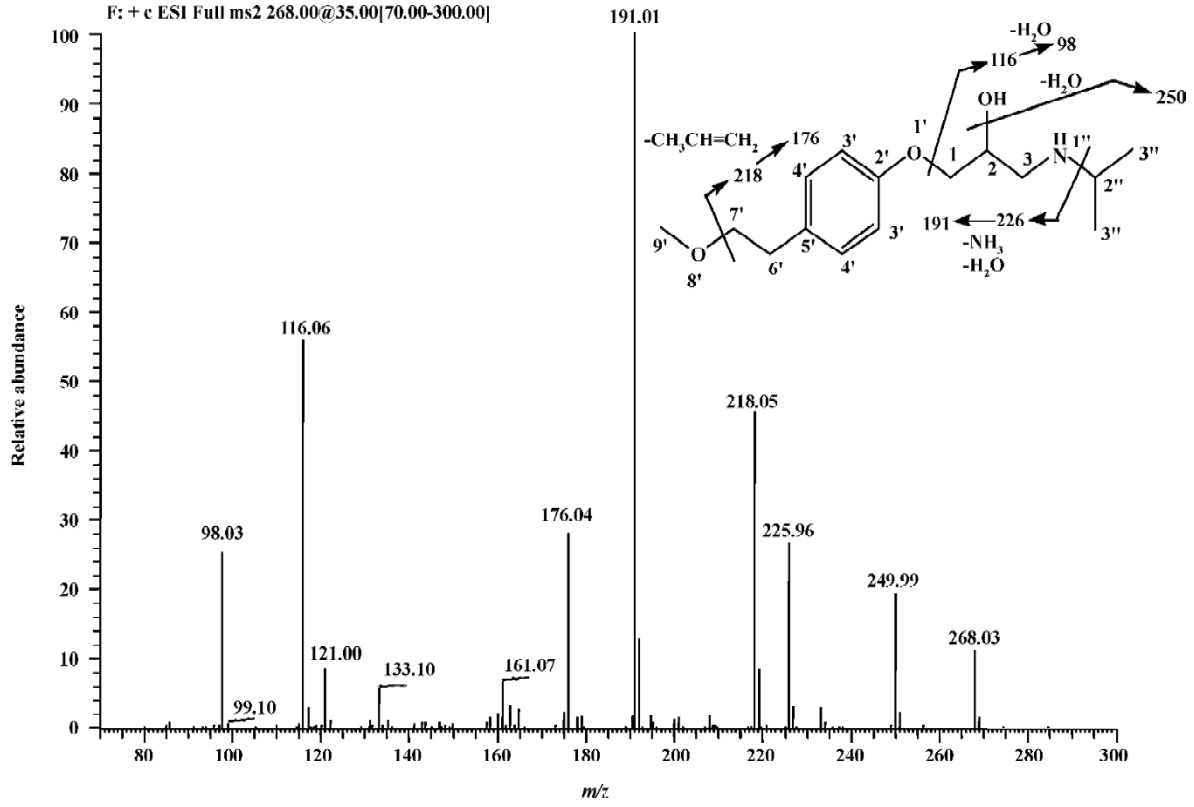

Figure 2. MS/MS spectra of metoprolol and its possible cleavage pathways. Only the abundant ions (>10%) were elucidated.

ied first. The MS/MS product ion spectra corresponding to the precursor ion of M0 at *m/z* 268 and its possible cleavage pathways are shown in Figure 2. The fragment ion at *m/z* 250 was 18 Da lower than that at m/z 268, which was due to the loss of H_2O, and the fragment ion at *m/z* 218 was formed by further neutral loss of CH_3OH, while the fragment ions at *m/z* 226 and *m/z* 176 were due to the loss of $CH_3CH{=}CH_2$ according to each precursor ions. The fragment at *m/z* 191 was due to neutral loss of NH_3 and H_2O based on the fragment ion at *m/z* 226. The fragment at *m/z* 116 was attributed to the elimination of the 4-(2-methoxyethyl)-phenoxy substituent from the structure, whereas the fragment ion at *m/z* 98 was associated with further loss of H_2O.

Metabolite M1 The retention time of M1 ($[M+H]^+$ at *m/z* 254) obtained by the LC/MS^n analysis was 7.57 min. The protonated molecule of M1 was 14 Da lower than that of M0,

and its fragment ions at *m/z* 236, 212, and 177 were also 14 Da lower than that at *m/z* 250, 226, and 191 of M0, respectively, while the ions at *m/z* 116 and 98 were the same as that of M0. This suggested that the 1-phenoxy-3-([1-methylethyl]amino)-2-propanol moiety was unchanged. Therefore, M1 was supposed as *O*-desmethylmetoprolol. To further verify its structure, NMR (^{1}H and ^{13}C) analysis was carried out and the results were as follows: ^{1}H NMR (D_2O): δ 0.96 (6H, t, J_1=5.28 Hz, J_2=4.99 Hz, H-3''), 2.62 (1H, m, H-2''), 2.69 (2H, t, J=6.68 Hz, H-6'), 2.75 (1H, m, H-3), 2.77(1H, m, H-3), 3.67(2H, t, J=6.66 Hz, H-7'), 3.87 (1H, m, H-2), 3.96 (2H, dd, J=5.28 Hz, 18.5 Hz, H-1), 6.85 (2H, d, J=8.30 Hz, H-3'), 7.12(2H, d, J=8.30 Hz, H-4'); ^{13}C NMR (D_2O): δ 23.6 ($C_{3''}$), 23.6 ($C_{3''}$), 39.6 ($C_{6'}$), 50.9 (C_3), 51.0 ($C_{2''}$), 65.4 ($C_{7'}$), 71.1 (C_1), 73.2(C_2), 117.6 ($C_{3'}$), 117.6 ($C_{3'}$), 132.9 ($C_{4'}$), 132.9 ($C_{4'}$), 134.6 ($C_{5'}$), and 159.4 ($C_{2'}$). Compared with the data of metoprolol in previous studies[12], M1 was finally identified as *O*-desmethyl-metoprolol.

Metabolite M2 The retention time of M2 ($[M+H]^+$ at *m/z* 268), obtained by the LC/MS^n analysis, was 5.87 min. The protonated molecule of M2 was the same as that of M0. The MS/MS spectrum of M2 showed product ions similar to those in M0, except for the absence of *m/z* 218 and 176, which indicated that changes took place in the methoxyethyl group. To further verify its structure, NMR (^{1}H and ^{13}C) analysis was carried out and the results were as follows: ^{1}H NMR (D_2O): δ 1.25 (6H, t, J_1=4.99 Hz, J_2=5.58 Hz, H-3''), 3.12 (1H, m, H-3), 3.21 (1H, m, H-3), 3.38 (3H, m, H-2'', 6'), 3.98 (1H, dd, J= 4.82 Hz, 9.91 Hz, H-1), 4.03 (1H, dd, J=3.73 Hz, 10.1 Hz, H-1), 4.18 (1H, d, J=4.45 Hz, H-2), 6.86 (2H, d, J=8.16 Hz, H-3'), 7.13 (2H, d, J=8.14 Hz, H-4'); ^{13}C NMR (D_2O): δ 20.6 ($C_{3''}$), 21.0 ($C_{3''}$), 46.2($C_{7'}$), 49.4(C_3), 53.8($C_{2''}$), 68.3(C_1), 72.2(C_2), 117.5 ($C_{3'}$), 117.5 ($C_{3'}$), 133.1 ($C_{4'}$), 133.1 ($C_{4'}$), 133.2 ($C_{5'}$), 159.2 ($C_{2'}$), and 184.0 ($C_{7'}$). The downfield shift of $C_{7'}$ from 65.4 to 184.0 ppm suggested there was a carboxy group in the structure of M2. Compared with the data of metoprolol in previous studies[12], M2 was finally identified as metoprolol acid.

Metabolites M3–M7 The precursor ion of M3 at *m/z* 284 was 16 Da higher than that of M0, and its fragment at *m/z* 207 was also 16 Da higher than that at *m/z* 191 of M0, while the same fragment ion at *m/z* 116 was in both the MS/MS spectra of M0 and M3, indicating that the 3-([1-methylethyl]amino)-2-propanol moiety was intact, hence, M3 was deduced as α-hydroxymetoprolol. The protonated molecule at *m/z* 226 (M4) and its fragment ion at *m/z* 74 were 42 Da lower than that of M0 and its fragment ion at *m/z* 116, respectively, while there was the same fragment ion at *m/z* 191, indicating that the metabolism took place at the 3-([1-methylethyl]amino)-2-propanol moiety. Furthermore, the protonated molecule at m/z 226 was identical to the fragment ion at *m/z* 226 of M0, and MS^3 product scan was performed on the fragment ion at *m/z* 226 of M0. The MS^3 spectrum was the same as that of MS^2 spectrum of M4, which gave further proof of the structure of M4. Many compounds were found to be *N*-dealkylated by CYP2D6[13], including other beta-1 selective aryloxy-propanolamine, such as propranolol[14] and atenolol[15]. *N*-dealkylation has become an established metabolic pathway, which could also occur on metoprolol. Hence, M4 was tentatively identified as *N*-desalkylmetoprolol. However, further investigation is needed.

The protonated molecule of M5 was at *m/z* 241; according to its fragmentation, M5 was supposed to be deaminated metoprolol. To obtain further information, the analysis was operated in negative ESI mode. A peak was detected with the deprotonated molecule at *m/z* 239. Following these results, M5 was finally identified as deaminated metoprolol. The protonated molecule of M6 at *m/z* 270 was 16 Da higher than that of M1. The fragment ions of M6 at *m/z* 252, 228, and 193 were also 16 Da higher than the fragment ions of M1 at *m/z* 236, 212, and 177, respectively, which suggested that M6 was a hydroxyl metabolite of M1. Similar metabolic pathways also exist in humans, dogs, horses, and rats[2,3].

For M7, the protonated molecule ($[M+H]^+$) was at *m/z* 416, and 162 Da higher than that of M1. The MS/MS spectra gave only 1 major fragment at *m/z* 254, which may be due to the neutral loss of glucose. Therefore, the MS^3 product scan was performed on *m/z* 254, and 3 major fragment ions at *m/z* 236, 212, and 177 were observed (data not shown), which was similar to the MS/MS spectra of M1. The retention time of M7 (5.05 min) was ahead of other metabolites, which was possibly due to its increasing polarity. These data suggested that M7 was the glucoside conjugate of M1. The eluate of M7 was collected and evaporated to dryness under a gentle stream of nitrogen. The residue was incubated with β-*D*-glucosidase (100 kU/L) at 37 °C for 24 h. The incubation solution was analyzed by LC/MS^n. The peak of M7 disappeared; instead, a peak with the same MS spectra and retention time as that of M1 was detected, which provided further evidence that M7 was the metabolite conjugated with glucoside.

Microbial transformation of metoprolol and comparison with mammalian metabolism In the present study, metoprolol was transformed by *C blakesleeana* AS 3.153 to 7 metabolites: *O*-desmethylmetoprolol (M1), metoprolol acid (M2), α-hydroxymetoprolol (M3), *N*-desalkylmetoprolol (M4), deaminated metoprolol (M5), hydroxyl-*O*-desmethylmetoprolol (M6), and glucoside conjugate of *O*-desmethylmetoprolol (M7). As shown in Figure 3, the structures of the metabolites and proposed biotransformation

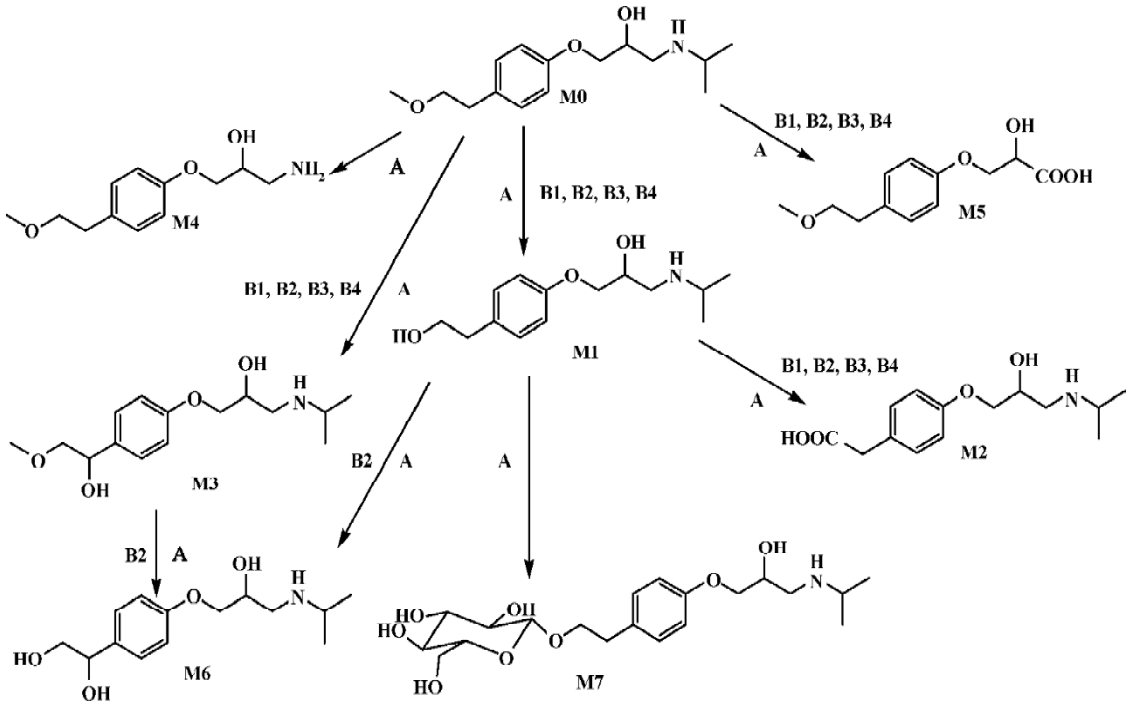

Figure 3. Proposed metabolic pathways of metoprolol transformed by *C blakesleeana* AS 3.153 and comparison with those in mammals. (A) *C blakesleeana* AS 3.153; (B) mammals: B1, humans; B2, horses; B3, dogs; B4, rats.

pathways by *C blakesleeana* are compared to those that have been identified in mammals[2,3]. After 120-h incubation by *C blakesleeana*, about 80% metoprolol was metabolized mainly to 3 metabolites, which was consistent with that in mammals. The yields of these metabolites were M1 (21.2%), M2 (59.4%), and M3 (4.5%), respectively.

Discussion

We reported a successful biotransformation of metoprolol by *C blakesleeana* AS 3.153 in this study. After centrifugation, the layer of mycelium was analyzed and trace amounts of metoprolol and its metabolites (M1 and M2) were detected. About 96% of the substrate and metabolites were obtained in the supernatant after centrifugation according to the initial amount of metoprolol. Thus, the analysis was performed only in the supernatant. After 120-h incubation in wheat-bran broth, about 80% of the drug was metabolized to 7 metabolites. As shown in Figure 3, 5 of the metabolites were essentially similar to those obtained in mammalian metabolism studies, whereas 2 novel metabolites, *N*-desalkyl-metoprolol and the glucoside conjugate of *O*-desmethyl-metoprolol, were identified. It has been well recognized that conjugation is an important metabolic pathway of many compounds both in mammals and in microorganisms[16–18]. A glucoside conjugate was detected in the present study, which conformed to previous studies that the glucosidation of drugs can be formed by microbial models[10,18,19]. In humans, metoprolol was metabolized to *O*-desmethylmetoprolol and metoprolol acid or α-hydroxymetoprolol, depending on the cytochrome P450 oxidation phenotype. The 3 primary metabolites of metoprolol in rats, dogs, and horses were *O*-desmethyl-metoprolol, metoprolol acid, and α-hydroxymetoprolol[2,3]. The fungus *C blakesleeana* converted metoprolol to 3 major metabolites, which showed similarities with the metabolism of metoprolol in mammals.

In conclusion, 7 metabolites of metoprolol were formed by *C blakesleeana* AS 3.153. The ability of *C blakesleeana* to mimic the mammalian metabolism and to perform novel biotransformations clearly demonstrated that microbial systems could predict potential routes of the mammalian biotransformation of drug candidates, and could also be used for the understanding of chemistry and biology significance of drug metabolism. Because of the capacity of the microbial metabolism, substrate concentrations used are much higher than those employed in other cell or tissue models and consequently allow for easier detection and isolation. Thus, the models can be scaled up easily for the preparation of metabolites for structure confirmation by NMR and further

pharmacological and toxicological studies.

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