Metabolic Mechanism of Delamanid, a New Anti-Tuberculosis Drug, in Human Plasma

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ABSTRACT

The metabolism of delamanid (OPC-67683, Deltyba), a novel treatment of multidrug-resistant tuberculosis, was investigated in vitro using plasma and purified protein preparations from humans and animals. Delamanid was rapidly degraded by incubation in the plasma of all species tested at 37°C, with half-life values (hours) of 0.64 (human), 0.84 (dog), 0.87 (rabbit), and 3.54 (rat). A major metabolite, (R)-2-amino-4,5-dihydrooxazole derivative (M1), was formed in the plasma by cleavage of the 6-nitro-2,3-dihydroimidazo(2,1-b)-oxazole moiety of delamanid. The rate of M1 formation increased with temperature (0–37°C) and pH (6.0–8.0). Delamanid was not converted to M1 in plasma filtrate, with a molecular mass cutoff of 30 kDa, suggesting that bioconversion is mediated by plasma proteins of higher molecular weight. When delamanid was incubated in plasma protein fractions separated by gel filtration chromatography, M1 was observed in the fraction consisting of albumin, γ-globulin, and α1-acid glycoprotein. In pure preparations of these proteins, only human serum albumin (HSA) metabolized delamanid to M1. The formation of M1 followed Michaelis-Menten kinetics in both human plasma and the HSA solution, with similar Km values: 67.8 μM in plasma and 51.5 μM in HSA. The maximum velocity and intrinsic clearance values for M1 were also comparable in plasma and HSA. These results strongly suggest that albumin is predominantly responsible for metabolizing delamanid to M1. We propose that delamanid degradation by albumin begins with a nucleophilic attack of amino acid residues on the electron-poor carbon at the 5 position of nitro-dihydroimidazooxazole, followed by cleavage of the imidazooxazole moiety to form M1.

Introduction

Delamanid (OPC-67683, Deltyba, nitro-dihydro-imidazooxazole derivative) is a new anti-tuberculosis (TB) drug for the treatment of multidrug-resistant tuberculosis. The in vitro metabolism of delamanid using human and animal liver microsomes has already been evaluated (Matsumoto et al., 2006). When delamanid was incubated with liver microsomes in the presence of reduced nicotinamide adenine dinucleotide phosphate, metabolites were nearly undetectable in the incubation mixture, suggesting that delamanid was not metabolized by cytochrome P450 enzymes. However, eight metabolites, including the abundant metabolite (R)-2-amino-4,5-dihydrooxazole derivative [DM-6705 (M1)], in human and animal plasma were detected and identified in the investigation for the in vivo pharmacokinetics and metabolism of delamanid, as described in the companion article (Sasahara et al., 2015) in this issue of Drug Metabolism and Disposition. The maximum plasma concentration of M1 was nearly half that of delamanid (0.32 μM versus 0.78 μM) following twice daily administration of 100 mg delamanid for 56 days (Gler et al., 2012; Shimokawa et al., 2014; Sasahara et al., 2015), suggesting that M1 is the major metabolite. On the basis of the chemical structure of M1, it is proposed that delamanid is cleaved directly at its 6-nitro-2,3-dihydroimidazo(2,1-b)-oxazole moiety by some extrahepatic mechanism (Matsumoto et al., 2006).

According to World Health Organization guidelines, a novel drug for drug-resistant TB should be used for long-term administration as an add-on therapy to at least three or more other anti-TB drugs to prevent the development of resistance (http://whqlibdoc.who.int/publications/2011/9789241501583_eng.pdf). It is important to identify the enzymes responsible for the metabolism of delamanid in humans. In the current study, biotransformation was first examined in animal and human plasma, and then the metabolic byproduct was identified by detection of radioactivity and simultaneous mass spectrometry (MS). The effects of temperature and pH on the formation of M1 and the rates of delamanid metabolism by various plasma protein fractions isolated by ultrafiltration and gel filtration were also investigated to identify the responsible enzymes. Finally, the kinetic parameters of M1 production were compared between human plasma and specific plasma proteins, which identified albumin as a major mediator of delamanid degradation. This is the first report describing the in vitro mechanism of delamanid metabolism in plasma.

Materials and Methods

Materials. 14C-delamanid, delamanid, and its metabolite M1 were obtained from Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). The chemical structure and labeled position of 14C-delamanid are shown in Fig. 1. The ABBREVIATIONS: AGP, α1-acid glycoprotein; DSA, dog serum albumin; HPLC, high-performance liquid chromatography; HSA, human serum albumin; LC-MS/MS, liquid chromatography-tandem mass spectrometry; M1, (R)-2-amino-4,5-dihydrooxazole derivative; MS, mass spectrometry; RSA, rat serum albumin; TB, tuberculosis.
specific radioactivity of 14C-delamanid was 4.14 MBq/mg and the radiochemical purity was 99.2%, as determined by high-performance liquid chromatography (HPLC). A detailed synthesis and spectral characterization of M1 are shown in the Supplemental Methods. Human plasma using heparin as an anticoagulant and human serum were prepared from three healthy males with approval of the Institutional Ethics Committee. Heparin plasma and serum from a male mouse (Institute of Cancer Research), rat (Sprague-Dawley), rabbit (New Zealand White), and dog (beagle) were supplied by Kitayama Labes Co. (Nagano, Japan). These animals were used as the preclinical species in pharmacology, pharmacokinetics, and toxicology studies for delamanid. Purified human serum albumin (HSA), essentially fatty acid free prepared from serum Fraction V (product number A1887), rat serum albumin (RSA) from Fraction V (A6272), canine serum albumin from Fraction V [dog serum albumin (FLSA), A9263], human γ-globulin (G4386), and α1-acid glycoprotein ([AGP], G9885] were purchased from Sigma-Aldrich Co. (St. Louis, MO). Other reagents were commercially available and of analytical grade.

Metabolism of Delamanid in Plasma. 14C-delamanid was dissolved in methanol at 2.07 MBq/0.5 mg per ml. The reaction mixture consisted of 14C-delamanid (5 μg/ml, 9.3 μM) and mouse, rat, rabbit, dog, or human plasma. The final solvent concentration was 1% (v/v). After preincubation of plasma at 37°C for 3 minutes, the reaction was started by adding 14C-delamanid (5 μg/ml). Incubation at 37°C was continued for 0, 0.5, 1, 2, and 4 hours. Nonlabeled delamanid (50 μg/ml) was also incubated at 37°C for 24 hours in mouse plasma to investigate the molecular structure of metabolites by MS.

Effects of Temperature and pH on Metabolite Formation in Plasma. 14C-delamanid (5 μg/ml) in human plasma was incubated at 25 and 0°C for 0, 1, 2, and 4 hours (in addition to at 37°C for 0, 0.5, 1, 2, and 4 hours). Further, 14C-delamanid (5 μg/ml) was incubated at 37°C for 0, 0.5, 1, 2, and 4 hours in 50 mM phosphate buffer (pH 6.0, 7.0, 7.5, and 8.0) containing 10% human serum. The final solvent concentration was 1% (v/v). After preincubation of plasma at 37°C for 5 minutes, the reaction was started by adding 14C-delamanid (5 μg/ml). Incubation at 37°C was continued for 0, 0.5, 1, 2, and 4 hours. Nonlabeled delamanid (50 μg/ml) was also incubated at 37°C for 24 hours in mouse plasma to investigate the molecular structure of metabolites by MS.

Metabolite Formation in Fractionated Plasma. Human plasma was centrifuged at 3000g for 30 minutes using a Centricon YM-30 (molecular mass cutoff of 30 kDa; Millipore Co., Billerica, MA). The plasma filtrate was incubated with 14C-delamanid (5 μg/ml) at 37°C for 0, 0.5, 1, 2, and 4 hours.

To obtain plasma protein fractions, high-performance gel filtration chromatography of human plasma was performed at room temperature using the columns TSK-gel G4000SWXL (7.8 mm i.d. × 300 mm, 8-μm particle size; Tosoh Co., Tokyo, Japan) and TSK-gel G3000SWXL (7.8 mm i.d. × 300 mm, 5-μm particle size; Tosoh Co.) in combination, 50 mM phosphate buffer (pH 7.0) as the mobile phase at 1 ml/min, and UV detection at 280 nm. After injection of 200 μl human plasma, the effluent was fractionated every 1 minute. Pure HSA (40 mg/ml), γ-globulin (12 mg/ml), and AGP (1 mg/ml) were also analyzed to confirm retention times. The eluate was adjusted to pH 7.5 with 1 N sodium hydroxide and incubated with 5 μg/ml of 14C-delamanid at 37°C for 8 hours. Delamanid was also incubated with HSA (40 mg/ml), γ-globulin (12 mg/ml), or AGP (1 mg/ml) in place of the eluate fraction containing all three proteins.

Kinetic Analysis on Metabolite Formation in Plasma and HSA. 14C-delamanid (10, 25, 50, 100, 250, and 500 μM in 2% dimethyl sulfoxide) was incubated at 37°C for 0.25 hours in human plasma or 40 mg/ml HSA (both in 50 mM phosphate buffer, pH 7.4). Total plasma protein concentration was determined using a Bio-Rad DC protein assay kit (Hercules, CA).

Metabolite Profiling in Plasma and Albumin. 14C-delamanid (5 μg/ml) was incubated in 50 mM phosphate buffer (pH 7.4) containing either 50% plasma or 20 mg/ml albumin from a rat, dog, and human at 37°C for 0, 0.5, 1, and 2 hours.

Binding of Delamanid to Serum and HSA. Degradation of delamanid is temperature dependent. To avoid the degradation of delamanid, the protein-binding studies were conducted at 20°C. The in vitro binding of 14C-delamanid (0.05, 0.5, and 5 μg/ml) to animal and human serum was determined by equilibrium dialysis for 4 hours (rabbit and dog serum) or 8 hours (rat, mouse, and human serum) using Spectrum/Por2 molecular porous dialysis membrane (Spectrum Laboratories, Inc., Rancho Dominguez, CA). The binding of 14C-delamanid (0.05, 0.5, and 5 μg/ml) to RSA, DSA, and HSA solutions (all at 40 mg/ml) was also determined. Further, the binding of 14C-delamanid (3 μM) to HSA (15 μM, 1 mg/ml) was determined in the absence and presence of the site-specific HSA-binding probes warfarin (Site I), diazepam (Site II), and digitoxin (Site III), all at 15, 75, and 150 μM. The dimethyl sulfoxide content was always ≤ 1% (v/v). The diazylated protein and dialysate were analyzed to determine the delamanid concentrations in bound and unbound fractions. After a scintillator cocktail was added to the sample, the radioactivity was determined by a liquid scintillation counter (LSC-3500; Aloka Co., Tokyo, Japan).

Sample Preparation for Radioactivity Counting and Mass Spectrometry. The reaction was terminated by mixing with two volumes of acetonitrile–formic acid (90:10, v/v). Following centrifugation at 21,800g for 5 minutes, 30 μl of the supernatant was analyzed by HPLC with simultaneous radioactive detection. Further, a scintillator cocktail (ACS II; Amersham Co., Biotics UK Ltd., Buckinghamshire, UK) was added to 30 μl of the supernatant, and the radioactivity determined by liquid scintillation (LSC-3500) to evaluate extraction and column recovery.

For measuring the metabolism of unlabeled delamanid in plasma, the reaction was terminated by mixing in an equal amount of acetonitrile, followed by centrifugation at 21,800g for 5 minutes and liquid chromatography–tandem mass spectrometry (LC-MS/MS).

HPLC and LC-MS/MS Procedures. To investigate the metabolism of labeled delamanid in plasma, two HPLC protocols were used. HPLC Method 1 used an LC-10A HPLC system (Shimadzu Co., Kyoto, Japan) equipped with a TSK-gel ODS-80Ts QA C18 column (4.6 mm i.d. × 150 mm, 5-μm particle size; Tosoh Co.) for sample analysis. The analyte was separated using a binary
solvent linear gradient with mobile phase A [water–acetic acid (100:1, v/v)] and B [acetonitrile–acetic acid (100:1, v/v): 0% B to 60% B from 0 to 35 minutes; 60% B to 90% B from 35 to 40 minutes at a flow rate of 1 ml/min; isocratic elution at 90% B from 40 to 45 minutes; and 0% B from 45 to 60 minutes. Before entering the radioactive flow detector, the column effluent was mixed in flow with a 1:2 scintillation cocktail (Ultima-Flo AP; PerkinElmer, Inc., Billerica, MA) pumped at a rate of 2 ml/min. The radioactivity in the effluent was monitored using a Radiomatic 525TR flow scintillation analyzer (PerkinElmer, Inc.). In HPLC Method 2, which was used to investigate the effects of temperature and pH, metabolite formation, kinetic analysis, and metabolite profiling in plasma and albumin were conducted using a model 2695 Alliance HPLC system (Waters Co., Milford, MA) equipped with an electrospray ionization interface introduced directly into a triple-quadrupole mass spectrometer (API3000; AB SCIEX, Foster, CA) equipped with an electrospray ionization interface operated in positive ion mode with the following operation parameters: gas temperature, 475°C; gas flow rate, 7 l/min; gas pressure, 70 psi; ion spray voltage, 4.5 kV; nebulizer gas, 12; curtain gas, 8; and collision gas, 8 (product ion scan only). Nitrogen was used in the ion source and the collision cell. A full scan and product ion scan were recorded within the mass range of m/z 100–600.

**Data Processing.** Data processing was performed using FLO-ONE version 3.65 (PerkinElmer, Inc.) in the flow scintillation analyzer. The radioactivity of delamanid metabolite in the sample was determined on the radiochromatogram, and the radioactivity was converted to equivalents of delamanid. The residual content of delamanid, metabolite formation, and other calculations were conducted with Microsoft Excel version 2003 (Seattle, WA). The half-life and Michaelis-Menten parameters [Michaelis-Menten constant (Km) and maximum velocity (Vmax)] were calculated using a nonlinear least-squares method by WinNonlin version 5.2 (Pharsight Co., St. Louis, MO). The intrinsic clearance (CLint) was obtained from Vmax/Km. Analysis of LC-MS/MS was performed using Analyst version 1.4.2 (AB SCIEX).

**Results**

**Metabolism of Delamanid in Plasma.** The degradation of 14C-delamanid during incubation in plasma at 37°C is shown in Fig. 2 and Table 1. Delamanid was rapidly degraded by incubation in human, dog, rabbit, mouse, or rat plasma at 37°C, with the shortest half-life in human plasma (0.64 hours), followed by dog (0.84 hours), rabbit (0.87 hours), mouse (1.90 hours), and rat (3.54 hours). Typical HPLC radiochromatograms of delamanid metabolites in plasma are shown in Supplemental Fig. 1. The major delamanid byproduct, M1, increased as substrate concentration decreased in plasma samples from all species.

The chemical structure of M1 was further investigated by LC-MS/MS analysis of mouse plasma containing nonlabeled delamanid. The mass spectra of the parent compound and the metabolite revealed protonated molecules ([M+H]+) at m/z 353 and 466, respectively, and a characteristic and intense fragment ion at m/z 352 in both positive product ion spectra (Fig. 3). The peak profile of the metabolite indicated the existence of a 4-[4-(4-trifluoro-methoxyphenox) piperidin-1-yl]phenoxymoieties without imidazooxazole. Additional fragment ions were observed at m/z 357 in spectra of the parent drug and at m/z 113, 288, and 449 in spectra of the metabolite. The
metabolite M1 was identified as (R)-2-amino-4,5-dihydrooxazole derivative by comparing the mass spectra and retention time of the product in the plasma sample to those of the authentic standard (Supplemental Fig. 2).

Effects of Temperature and pH on Metabolite Formation in Plasma. The rates of M1 formation in human plasma incubated at various temperatures are shown in Fig. 4. The biotransformation to M1 after 4 hours was 51.7% at 37°C and 36.3% at 25°C, whereas no M1 was detected after 4 hours at 0°C.

Metabolism was also highly pH dependent (Fig. 5). After 4 hours at 37°C in 10% human plasma, M1 formation was 0.0% at pH 6.0, 4.8% at pH 7.0, 12.7% at pH 7.5, and 20.1% at pH 8.0. In contrast, M1 was not formed during incubation for 4 hours in 50 mM phosphate buffer at any pH in the absence of plasma (data not shown).

Metabolite Formation in Fractionated Plasma. Delamanid was not converted to M1 in the filtrate of human plasma obtained with a molecular mass cutoff of 30 kDa, indicating that metabolism required the presence of plasma proteins of a molecular mass ≥ 30 kDa. When delamanid degradation was examined in plasma fractions separated by gel chromatography, M1 was observed in the fraction containing albumin, γ-globulin, and AGP (Fig. 6). In the presence of HSA, delamanid was metabolized to M1, whereas no M1 was detected following delamanid incubation with γ-globulin or AGP. Thus, metabolism requires HSA.

Kinetic Analysis on Metabolite Formation in Plasma and HSA. The total protein concentration in human plasma samples was approximately 80 mg/ml. Several concentrations of delamanid were incubated in human plasma or 40 mg/ml HSA. The formation of M1 followed Michaelis-Menten kinetics in both human plasma and HSA (Fig. 7). The Eadie-Hofstee plot for the formation of M1 in plasma showed a monophasic profile. The $K_{in}$, $V_{max}$, and $CL_{int}$ values for plasma were 67.8 μM, 7.55 pmol/min per mg, and 0.111 μl/min per mg, respectively, and the values found in HSA alone were 51.5 μM, 11.7 pmol/min per mg, and 0.227 μl/min per mg, respectively (Table 2).

Metabolite Profiling in Plasma and Albumin. In addition to the kinetic profile, the metabolic pattern of delamanid in 50% plasma was similar to that in 20 mg/ml albumin (Fig. 8). The degradation rates of delamanid were highest in human plasma and HSA, followed by dog and rat plasma and albumin. The residual content of delamanid after 1 hour at 37°C in 50% human plasma was 52.8%, substantially higher than in dog (82.6%) and rat (93.5%), whereas the corresponding biotransformation rate to M1 was highest in 50% human plasma (21.0%), followed by dog (6.5%) and rat (3.1%). Similarly, the residual delamanid content was lower after incubation (1 hour at 37°C) in 20 mg/ml HSA (50.1%) compared with that in DSA (77%) and RSA (92.0%), and the corresponding M1 formation was highest in HSA (19.9%), followed by DSA (9.7%) and RSA (5.2%).

Binding of Delamanid to Serum and HSA. The in vitro protein binding ratio was ≈ 99.3% in all serum samples at all $^{14}$C-delamanid concentrations tested (Table 3), whereas binding was ≈ 97.4% to RSA, DSA, and HSA. The binding of $^{14}$C-delamanid to HSA was not changed in the presence of the Site I–specific binding probe warfarin, Site II–specific probe diazepam, or Site III–specific probe digitoxin (Table 4). The radiochemical purity of delamanid incubated at 20°C in human serum and HSA was more than 86%, suggesting that delamanid was stable in the protein-binding studies.

Discussion

When delamanid was incubated in human plasma at an appropriate temperature and pH, the metabolite M1 increased in parallel with a decrease in the substrate concentration, indicating that M1 is a primary producer of plasma-mediated degradation. M1 was also the
CL is likely responsible for the metabolism of delamanid. The constitutes about half of total plasma protein (40 mg/ml of 80 mg/ml), human plasma (Table 2), suggesting that plasma albumin, which (11.7 pmol/min per mg and $0.227 \text{ml/min per mg}$) were comparable to those in 40 mg/ml HSA (11.7 pmol/min per mg and 0.227 $\mu$mol/min per mg of albumin). In contrast, no delamanid metabolism was observed following incubation with the other two high molecular weight proteins in the original HSA fraction: γ-globulin and AGP (Fig. 6). The purity of the commercial albumin employed in the in vitro studies was > 96%, with the remaining being mostly globulins (quality A-1887; Sigma-Aldrich Co.). Although hydrolase (mainly pseudo-cholinesterase) contamination of the purified HSA preparation cannot be completely excluded, metabolism was also observed by recombinant human albumin (product number A7223; Sigma-Aldrich Co.). This result and the similarity in kinetics between HSA and plasma strongly suggest that delamanid was metabolized predominantly by albumin in plasma.

The in vitro biotransformation of delamanid increased in a pH-dependent manner (Fig. 5), and delamanid did not degrade at pH 6.0. Considering that the pKa of delamanid is approximately 4.3, the pH sensitivity suggests that the pKa value of the catalytic amino acid residue(s) in plasma albumin may be important for the reaction. The metabolic patterns of delamanid in dog and rat plasma were also similar to those in dog and rat albumin (Fig. 8), suggesting that plasma albumin is predominantly responsible for delamanid metabolism in the rat and dog as well. Protein binding to delamanid was also similar to that in humans (Table 3). Nonetheless, the degradation rate of delamanid was highest in the human plasma and albumin solution, followed by dog and rat. It was reported that the hydrolytic degradation of Boc5 in plasma was mediated by serum albumin, and that species differences in hydrolysis could be attributed to variations in the albumin sequence and high-order structure across species (Ge et al., 2013). The species differences in the degradation rate of delamanid may thus also stem from species variation in the sequence of albumin.

Although extraction recovery and HPLC column recovery were favorable, the rate of delamanid degradation was higher than the rate of M1 formation in both plasma and albumin, suggesting that M1 is the major but not the only metabolic byproduct. These other byproducts may include the minor metabolites observed at retention times from 24 to 30 minutes (Supplemental Fig. 1), which remain to be identified and characterized.

In a novel biotransformation, M1 was uniquely formed by cleavage of the 6-nitro-2,3-dihydroimidazo(2,1-b)-oxazole moiety of delamanid in plasma albumin. On the basis of the fact that the authentic standard M1 is directly synthesized from delamanid and alkaline reagents, such as 25% ammonia solution or alkylamines (Supplemental Fig. 2), basic amino acid residues, such as lysine or arginine, in albumin may be important for the metabolism of delamanid. The proposed degradation mechanism of delamanid by albumin is illustrated in Fig. 9. Because of the electron withdrawing property of the neighboring nitro group, the electron-poor C-5 of the delamanid 6-nitro-2,3-dihydroimidazoo (2,1-b)-oxazole moiety can react easily with a nucleophile. When amino acid residues in HSA attack this carbon, an albumin-delamanid adduct is produced. The delamanid adduct is further hydrolyzed in the presence of water, resulting in the primary metabolite M1. However, further work is necessary to resolve the details of the mechanism.
long- and short-chain fatty acid esters (Wolfbeis and Gürakar, 1987). Among previous studies, the most relevant example of albumin-catalyzed metabolism to this study is that of \( N \)-trans-cinnamoyl imidazoles (Ohta et al., 1983). It appears that this interaction involves fast acylation of albumin to form cinnamoyl albumin, followed by a slow deacylation of cinnamoyl albumin. The electron-withdrawing substituent, the carbonyl group (C=O) of \( N \)-trans-cinnamoyl imidazole, facilitates the acylation.

The electron-poor carbon at the C-5 position of the delamanid imidazo-oxazole structure is also able to react with a nucleophile. Considering the fact that a delamanid analog without the nitro group was not metabolized by HSA (data not shown), an electron-withdrawing nitro group of delamanid is suggested to be important for the propensity toward ring scission by albumin. Ohta et al. (1983) proposed that acylation by albumin occurs at a reactive residue of the R site (Tyr-411), which corresponds to Sudlow’s Site II (Sudlow et al., 1976; Ozeki et al., 1980; Salvi et al., 1997). The nucleophilic character of Tyr-411 for the esterase-like activity toward \( p \)-nitrophenyl esters suggested that nucleophilic attack by albumin on the substrate results in an acylated albumin derivative that is then deacylated by a general acid or base catalysis (Sakurai et al., 2004). Accordingly, a study using \( p \)-nitrophenyl acetate as a substrate showed that the enzymatic activity of HSA was dependent on the presence of Tyr-411 (Watanabe et al., 2000). For the protein bindings, at least three binding sites, Site I, Site II, and Site III, are reported to be present on HSA. The saturation of the protein-binding capacity of delamanid to HSA (15 \( \mu \)M) was not observed at high concentrations (up to 30 \( \mu \)M; data not shown). Further, the protein binding of delamanid in HSA was not affected by varying the concentrations of Site I–III specific probes (warfarin, diazepam, and digitoxin, respectively) (Table 4). These results suggest that delamanid may bind nonspecifically to HSA. The effects of inhibitory protein binding ligands on delamanid metabolism require further study to clarify the molecular mechanisms of albumin-mediated metabolism.

Esterase-like activity of HSA on olmesartan medoxomil hydrolysis has also been reported (Ma et al., 2005). Chemically modified HSA derivatives (Tyr, Lys, His, and Trp modifications) and the mutant HSAs K199A, W214A, and Y411A exhibited significantly lower reactivity, suggesting that (wild-type) Lys-199, Trp-214, and Tyr-411 play important roles in hydrolysis. Moreover, using selective amino acid reagents, these authors concluded that Cys, Trp, Arg, and Tyr participate in the carbarylase activity of HSA (Sogorb et al., 2004). Finally, it was reported that the bioconversion of aspirin by albumin is a pseudo-esterase reaction in which aspirin stably acetylates lysines on albumin and releases salicylate (Liyasova et al., 2010). These reports collectively suggest that amino acid residues, such as lysine, tryptophan, and arginine, phenolic hydroxyl groups, such as tyrosine, and thiol groups, such as cysteine in HSA, may be involved in the first step of delamanid metabolism by albumin.

The overall in vivo metabolism of delamanid was qualitatively similar across species, including humans and the predominant preclinical study species. However, quantitative differences were observed among species (Sasahara et al., 2015). For instance, M1 concentration after repeated administration was much higher in a human and dog than in rodents, consistent with the more rapid

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### Table 4

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<thead>
<tr>
<th>Probe</th>
<th>Concentration (( \mu )M)</th>
<th>Delamanid Protein Binding (%)</th>
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<tr>
<td>Control</td>
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<tr>
<td></td>
<td>15</td>
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<td></td>
<td>150</td>
<td>98.0 ± 0.4</td>
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![Proposed degradation mechanism of delamanid by albumin.](image-url)
formation of M1 in human and dog plasma in vitro (Fig. 2; Table 1). As M1 formation appears to be the primary metabolic reaction of delamanid, it is the determinant of the interspecies differences in delamanid biotransformation.

In conclusion, the new anti-TB drug delamanid is metabolized to the (R)-2-amino-4,5-dihydrooxazole derivative (M1) by albumin in plasma. The degradation of delamanid by albumin is proposed to begin with an attack by amino acid residues of albumin on the electron-poor carbon at the 5 position of nitro-dihydro-imidazoazole, followed by cleavage of the imidazoazole molety to M1.

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Authorship Contributions

Participated in research design: Shimokawa, Sasahara, Koyama, Kitano, Shibata, Yoda, Umehara.

Conducted experiments: Shimokawa, Sasahara, Koyama, Kitano, Shibata, Yoda.

Contributed new reagents or analytic tools: Shimokawa, Sasahara, Koyama, Kitano.

Performed data analysis: Shimokawa, Sasahara, Koyama, Kitano, Shibata, Yoda.

Wrote or contributed to the writing of the manuscript: Shimokawa, Sasahara, Kitano, Umehara.

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