Poster #942

Human Disposition and Metabolism of Orally Administered ([14]C)ABT-773

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ABSTRACT

Background: A study was undertaken to characterize the metabolic fate of the new novel ketolide, [14C]ABT-773, following oral dosing in man.

Methods: After the administration of an oral 150 mg (128 µCi) [14C]ABT-773 dose to six healthy male subjects, total radioactivity was measured in urine, feces and blood. Urine and plasma radioactivity were measured directly by liquid scintillation analysis. Metabolites were estimated in the samples using HPLC flow scintillation analysis.

Results: The peak mean plasma concentration of [14C]ABT-773-derived radioactivity was seen at 5 hours post-dose (0.242 µg eq/g), with concentrations falling below detection by 48 hours. The concentrations in plasma were much higher than in blood, indicating that [14C]ABT-773-derived radioactivity does not significantly partition into red blood cells.

Recovery of radiolabeled ABT-773 was nearly complete by 168 hours post-dose, averaging 94.2%. Fecal excretion was the major elimination route, averaging 87.2% of the dose. Urinary recovery averaged 7.0%. Over 90% of the total urinary and fecal radiolabel was eliminated by 24 hours and 96 hours, respectively. Unchanged ABT-773 was recovered in the urine, accounting for 90% of urinary radioactivity. Only the N-desmethyl metabolite (M1) was observed in urine, representing the remaining 10%.

Evidence of extensive metabolism (7 metabolites) was observed in the feces. M1 was the major component (34.7% of the dose) while ABT-773 averaged 31.1% of the dose. Smaller amounts of a 10-hydroxy-product (M3), a N-desmethyl-10-hydroxy-product (M4) and an unidentified oxidative product (M6) accounted for 5.8, 4.2, and 3.5% of the administered dose. Three other peaks accounted for 4.2% of the dose.

Conclusions: Oxidative metabolism is the primary elimination route of [14C]ABT-773 in humans, accounting for over 50% of the administered dose.

INTRODUCTION

ABT-773 is novel ketolide antimicrobial agent with activity against penicillin- and macrolide-resistant gram positive bacteria (Figure 2). *In vitro* studies in human liver fractions identified four metabolites mediated principally by cytochrome P450 3A4.[1] *In vivo* disposition studies in non-human primates suggested that extensive metabolism via oxidation and subsequent hepatobiliary excretion was the principle route of elimination.[2] This study was undertaken to characterize the metabolic fate of ABT-773 in humans.

METHODS

- The human study and sample collection was conducted by Covance Laboratories, Madison, WI. Samples were shipped to Abbott Laboratories for completion of analyses.
- Samples were processed as outlined in Figure 1. Urine samples were concentrated by loading on C_8 solid phase cartridges (Isolute, IST) and eluted with 100% methanol. Analyses were performed on a liquid chromatography system consisting of a quaternary pump, an auto sampler and a diode array detector set at 251 nm (Agilent Technologies 1100 System). Plasma samples were loaded directly on to reverse-phase extraction cartridges (OASIS HLB, Waters) washed with 3-5 column volumes of water and eluted with 100% methanol. Recoveries for urine and plasma averaged greater than 90%.
- Separations were achieved at room temperature with a Beckman Ultrasphere 5 micron (250 x 4.6) C_{18} column. A linear gradient of 20-40% acetonitrile in buffer (50 mM ammonium acetate, pH adjusted to 4 with formic acid) over 60 minutes was used as column eluant at a flow rate of 1 mL/minute.
- For urine and feces, radioactivity [counts per minute (CPM)] in the column effluent was monitored with a Flo-One/Beta Model A-500 radioactivity detector (Packard Instruments) equipped with a 0.25 mL cell and a ratio of column effluent to liquid scintillation cocktail (Ultima-Flo™ M) of 1:3. For plasma, column eluant was collected into 96-well solid scintillant plates (Deepwell LumaPlate, Packard Instruments) using a 96-well plate fraction collector (FC204, Gilson). Plates were then centrifuged under vacuum overnight until dry (AES2010 AES, Savant). Radioactivity was measured using a low-background 96-well plate counter (TopCount®, Packard Instruments).
- Metabolites were identified by LC-MS (Perkin-Elmer Sciex API 300 Tandem Mass Spectrometer) and/or NMR.
- Pharmacokinetic parameters of ABT-773 were estimated by semi-parametric methods using a set of macro routines in a spreadsheet (Pharmkin/MS Excel).

Figure 1. Methods-Sample Processing

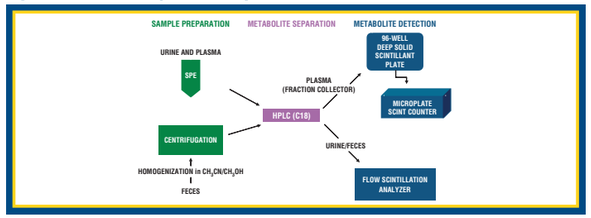


Figure 2. Proposed Human *In Vivo* Major Metabolic Pathways for [14C]Abbott-195773

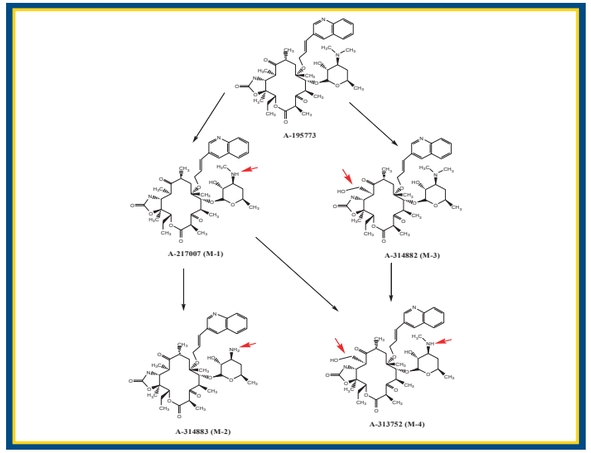


Table 1. Excretion of Radioactivity in Human Urine and Feces After Administration of [14C]Abbott-195773

	Percent of Total Dose Radioactivity		
Subject ID	Urine (0-168 hr.)	Feces (0-168 hr.)	Total Recovery
R010	6.9	91.4	98.3
R020	13.0	83.7	96.7
R030	7.5	79.6	87.1
R040	4.4	92.2	96.6
R050	5.1	90.2	95.3
R060	5.0	85.9	90.0
Average	**7.0**	**87.2**	**94.2**

Figure 3. ABT-773 and Its Major Metabolite Distribution in Feces After Single Oral Dose

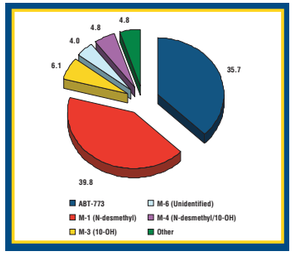


Figure 4. ABT-773 and Its Major Metabolite Distribution in Urine After Single Oral Dose

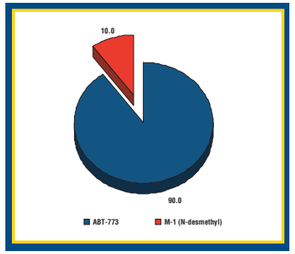


Figure 5. [14C]Abbott-195773 Metabolic Profile in Human After Single Oral Dose

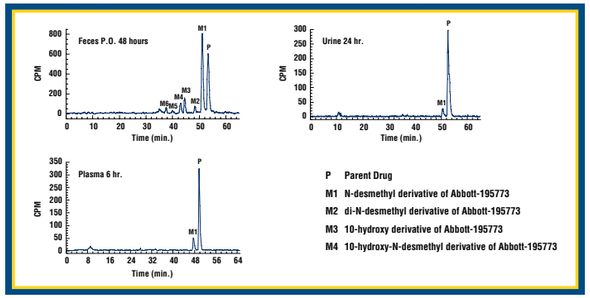


Table 2. Pharmacokinetic Parameters of [14C]ABT-773 Following a Single 150 mg Oral Dose

	M-1	[14C] ABT-773 150 mg (n=6)	ABT-773 PK 150 mg (n=12)
AUC (ng•hr/g)	429.30	1724	1873
$T_{1/2}$ (hr)	4.6	4.2	5.7
Cl/F (L/h)		87	92
T_{max} (h)	5.0	6.0	1.5
C_{max} (ng/g)	51.0	194.0	439
k (hr^{-1})	0.150	0.167	
Cl_f/Cl_m (AUC_m^2/AUC_p^2)		0.25	

Figure 6. ABT-773 Parent and Metabolite Levels in Human Plasma After Single Oral Dose

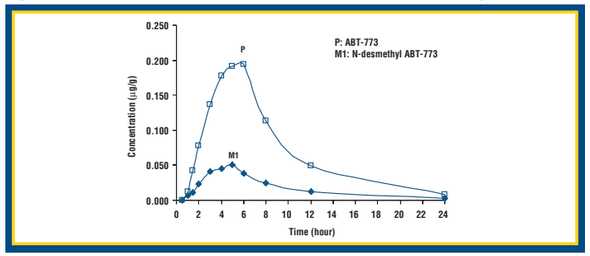
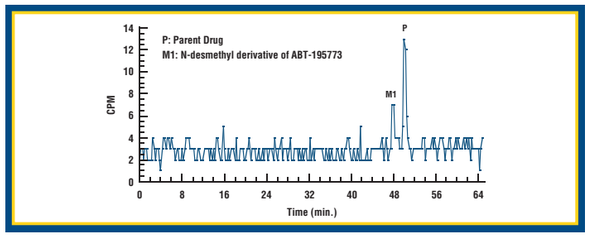


Figure 7. Radioactivity from [14C]ABT-773 in the 1-Hour Pooled Human Plasma Sample Using a Solid Scintillant 96-Well Plate Method



SUMMARY

- Based on this study and previous work, a sequential metabolic pathway for ABT-773 in humans is proposed (Figure 2).
- After dosing [14C]ABT-773, 94.2% of total dose radioactivity was recovered from urine and feces after 168 hours (Table 1). The major route of elimination in humans was metabolic with subsequent hepatobiliary elimination (Figures 3 and 4).
- The only metabolite detected in human urine and plasma was the N-desmethyl product of ABT-773 (M1). In feces samples, M1 was also the major metabolite. In addition, other oxidative products (M2, M3 and M4) were also detected (Figure 5).
- The disposition variables were comparable to parent disposition studies using non-radiolabeled compound (Table 2).
- ABT-773 was the major component circulating in plasma. The N-desmethyl metabolite was the only circulating metabolite detected and exhibited formation-rate limited kinetics (Figure 6). Therefore, no excessive accumulation of metabolite is expected to occur.
- Due to the very low plasma concentration of radioactivity, the parent and metabolite of ABT-773 could not be detected by using flow scintillation analysis. However, identification of circulating plasma metabolites was possible by pooling time points from all subjects and by using a sensitive microplate solid scintillation method. Using this method total radioactive counts of 60 cpm were discernable (Figure 7).

REFERENCES

1. Hernandez L, Sadrzadeh N, Krill S, Ma Z, Marsh K. Preclinical pharmacokinetic profile of ABT-773 in mouse, rat, monkey and dog (poster #2148). Presented at the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, September 26-29, 1999.
2. Guan Z, Jayanti V, Johnson M, Nequist G, Reisch T, Anderson L, Everitt E, Roberts E, Schmidt J, Rotert G, Surber B, Thomas S, Rodriguez C, Lee R, Kumar G, Roberts S, Lin J. *In vitro* and *in vivo* metabolism of [14C]ABT-773 (poster #2149). Presented at the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, September 26-29, 1999.