Absorption, Distribution, Metabolism and Excretion of [^{14}C]-Bemnifosbuvir in Rats

SAT-411

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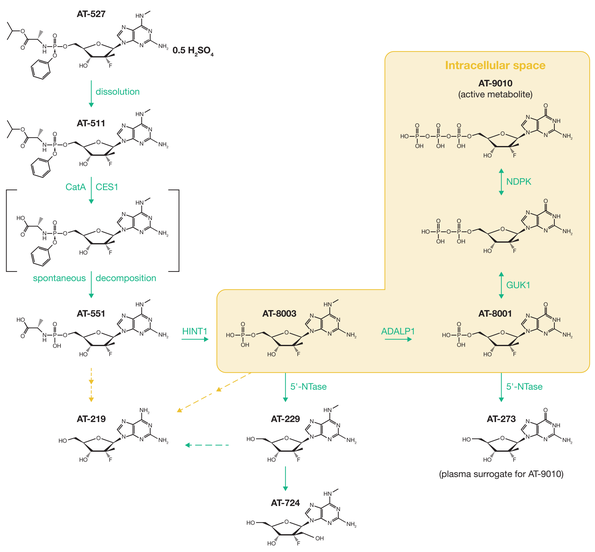
INTRODUCTION

- Bemnifosbuvir (BEM, AT-527), an oral prodrug of a 6-modified guanosine nucleotide analog, is currently in development for the treatment of patients with coronavirus disease 2019 (COVID-19) or chronic hepatitis C virus (HCV) infection
- BEM is a hemisulfate salt of AT-511, a phosphoramidate protide that is converted after multistep activation to the active 5'-triphosphate (TP) metabolite AT-9010, a potent inhibitor of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and HCV replication
- BEM was readily absorbed when dosed orally in preclinical species; it distributed into tissues and underwent a multistep metabolic activation pathway to its active triphosphate[1]
- The activation pathway (**Figure 1**) involves sequential hydrolysis of the carboxyl ester moiety catalyzed by human cathepsin A (CatA) and/or carboxylesterase 1 (CES1) to form AT-551 (the L-alanyl metabolite of AT-511), which is then subject to cleavage of the amino acid moiety by histidine triad nucleotide-binding protein 1 (HINT1), thus forming AT-8003 (the monophosphate [MP] of AT-229)
- Adenosine deaminase like protein 1 (ADALP1) subsequently converts AT-8003 to AT-8001 (the MP of the guanosine analog), which is further anabolized to the diphosphate by guanylate kinase 1 (GUK1) and ultimately to the pharmacologically active 2'-fluoro-2'-C-methylguanosine TP (AT-9010) by nucleoside diphosphate kinase (NDPK)
- AT-9010 can be dephosphorylated to its MP, AT-8001, and both AT-8003 and AT-8001 can be dephosphorylated by 5'-nucleotidase (5'-NTase) to their respective nucleosides AT-229 and AT-273

Figure 1. Proposed metabolic and activation pathway



METHODS

- The study utilized four groups of male SD rats (Groups 1–3, 5) and one group of male Long-Evans (LE) rats (Group 4). Groups 1 and 2 were used for evaluation of excretion mass balance in intact and bile duct cannulated (BDC) rats, respectively; Group 3 was used for evaluation of plasma total radioactivity pharmacokinetics; and Groups 4 and 5 were used for evaluation of plasma total radioactivity and tissue distribution of total radioactivity using quantitative whole-body autoradiography (QWBA)
- All rats received a single oral dose of [^{14}C]-AT-527 at 60 mg/kg as free base and a target radioactive dose of ~200 µCi/kg using a dose volume of 3 mL/kg. The dosing formulation was prepared as a solution of [^{14}C]-AT-527 and AT-527 in Milli-Q water (vehicle)
- Group 1 provided urine, feces, and cage residue samples through 168 h post-dose. Group 2 provided bile, urine, feces, and cage residue samples through 96 h post-dose. Group 3 provided plasma samples at predose (0), 0.5, 1, 2, 4, 8, 24, and 48 h post-dose
- Excreta, cage residue, plasma, and dosing formulation samples were analyzed by liquid scintillation counting. Plasma samples and carcasses for QWBA analysis were collected at 1, 2, 4, 8, 24, 48, 72, 168, 528, and 840 h post-dose for Group 4 and at 2, 24, and 168 h post-dose for Group 5 (one rat per time point)

RESULTS

Figure 2. Cumulative excretion of total radioactivity in intact male SD rats (A) and BDC rats (B)

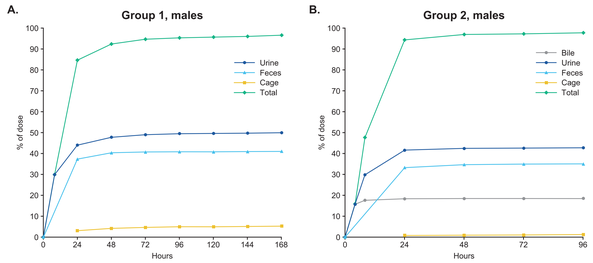


Table 1. Excretion of BEM and metabolites recovered in SD intact and BDC rats

Elimination route	Recovery (% dose of radiolabel, mean ± SD)	
	Group 1 (intact, n=3)	Group 2 (BDC, n=3)
Bile	NA	18.6 ± 3.7
Urine	50.1 ± 7.1	42.8 ± 2.0
Feces	41.0 ± 6.3	35.1 ± 3.2
Cage	5.53 ± 2.25	1.40 ± 0.57
Total	96.7 ± 0.34	97.8 ± 0.7

Cumulative recovery data over 168 h (Group 1) and over 96 h (Group 2).
BDC, bile-duct cannulated; NA, not applicable; SD, Sprague-Dawley.

- About 97% of the labeled BEM was recovered primarily in urine and feces

Metabolism

Figure 3. Individual and mean plasma total radioactivity concentration-time profiles in Group 3 male SD rats

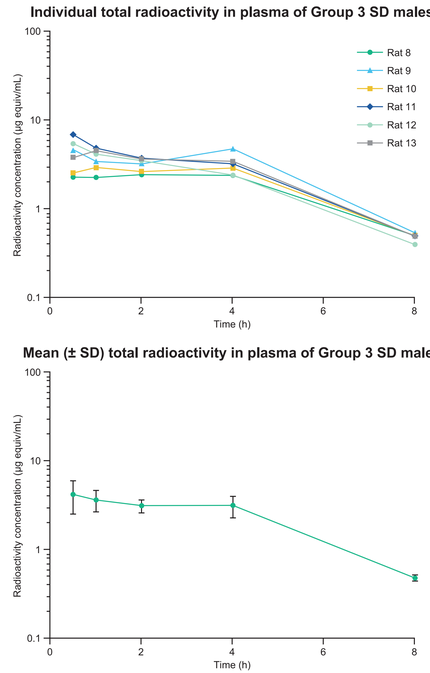


Table 2. Metabolic profile of BEM and its metabolites in rats

Component	% AUC	% Dose						
	Plasma[a]	Urine		Bile	Feces		Cumulative excreta	
Time postdose	0–8 h	0–72 h	0–48 h	0–24 h	0–48 h			
	Group 3	Group 1	Group 2	Group 2	Group 1	Group 2	Group 1	Group 2
AT-511	MS	MS	MS	MS	MS	MS	---	---
AT-551	6.08	0.363	1.05	14.0	MS	MS	0.36	15.05
AT-229	82.5	34.3	29.8	0.0746	28.1	26.3	62.40	56.17
AT-273	1.49	7.83	6.58	MS	4.48	2.73	12.31	9.31
AT-219	2.59	2.13	2.50	0.0257	0.409	0.323	2.54	2.85
AT-8003	MS	MS	MS	MS	ND	ND	---	---
M329_2	1.53	0.895	0.573	0.0231	MS	MS	0.90	0.60
M329_3	0.874	0.405	0.234	0.139	ND	ND	0.41	0.37
M329_4	MS	0.189	0.350	0.161	1.15	0.486	1.34	1.00
M450	NA	MS	0.350	0.200	ND	ND	---	0.55
M489_1	1.80	0.171	MS	1.93	0.994	1.25	1.17	3.18
M489_2	ND	2.46	0.657	0.273	1.59	MS	4.05	0.93
SUM % dose[b]	–	49.1	42.6	18.5	40.4	34.8	89.5	95.9

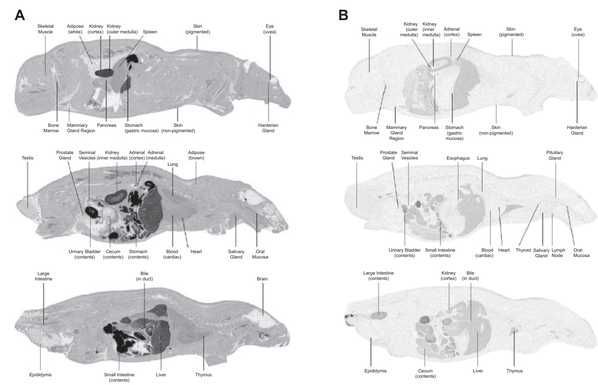
MS, below quantification limit on radiochromatogram but detected by mass spectrometry; NA, not applicable (AUC could not be calculated with less than three consecutive and measurable concentrations); ND, not detected.
Group 2 were bile-duct cannulated rats; Groups 1 and 3 were intact rats.
[a]Plasma data are expressed as % AUC. SUM = 100% AUC;
[b]This SUM included a few metabolites detected but with unknown structures (not shown).

- The main BEM metabolites detected – AT-551, AT-229, AT-273, AT-219 – were consistent with the proposed metabolic and activation pathway (**Figure 1**)

QWBA tissue distribution

- The high concentration of radiolabel in tissues such as kidney and liver was not retained at 24 h post-dose, reflecting the elimination half-life of <18 h for most tissues

Figure 4: QWBA of the radioactivity distribution in a male Long-Evans rat at 1 h (A) and 24 h (B) following a single 60 mg/kg oral dose of [^{14}C]-AT-527



CONCLUSIONS

- Drug-derived radioactivity was widely distributed throughout the body in the rats, with quantifiable concentrations present in many tissues through 24 h
- The primary route of elimination was in the urine (43%) and feces (35%). Approximately 19% of the radiolabel was recovered in the bile, suggesting a potential bioavailability of >60% of BEM following oral administration in the rat
- Detected metabolites were in accordance with the proposed metabolic and activation pathway
- High tissue concentrations of radiolabel were observed in kidney, liver, thymus and small intestine while relatively low concentrations were measured in the brain, spinal cord, bone and eye lens

References

1. Good SS, et al. PLoS One 2020;15:e0227104.

Acknowledgements

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Disclosures

All the authors are employees of Atea Pharmaceuticals.

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