Plasma and tissue disposition of AT-527 and its active triphosphate metabolite in monkeys: implications for dose selection for patients with COVID-19

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Background

AT-527 is a novel guanosine nucleotide produg with potent in vitro antiviral activity against HCV and human coronaviruses including SARS-CoV-2 (EC_{50}=0.5 μM), the virus responsible for COVID-19. The safety and efficacy of AT-527 demonstrated in clinical trial HCV subjects prompted clinical evaluation of the drug candidate in subjects with COVID-19.

Methods

Tissue distribution PK in non-human primates (NHP)
- Animals: Male non-native cynomolgus monkeys, >2 yrs old and 2 kg BW (Hainan Jangang Laboratory Animal Co., Ltd., Hainan, China), were group housed during the 5-day acclimation period or individually housed during the study, provided ad libitum access to RC water, fed once daily.
- Treatment: AT-527 was dosed in a suspension via oral gavage to 12 monkeys according to the following BID regimen: 60 mg/kg loading dose followed by 30 mg/kg every 12 h for 3 days. This regimen approximated intermittently to a human regimen of 1100 mg LD + 550 mg BID.
- Blood and tissue sampling
  - Blood samples (0-5 ml) were collected from 3 animals prior to and post the 5th dose, 0, 1, 2, 4, 6, 8, and 12 h, and at sacrifice (2 h after the last dose). Blood samples were collected from the rest of the animals, in groups of three, at 12, 24 and 48 h after the last dose, before they were sacrificed for tissue. Plasma was then obtained and prepared for LC/MS/MS analysis.
  - After the terminal blood collection, animals were anesthetized and duplicate samples (∼1 g) of lung, liver and kidney tissue were collected from each animal at the same organ location, snap frozen in liquid nitrogen, and stored at −80°C. Portions of the frozen tissues were then homogenized and extracted for LC-MS/MS analysis.

In vitro formation of AT-510 in human and monkey hepatocytes
- Plate cryopreserved hepatocytes from humans (mixed gender, post of 10 donors) and male cynomolgus monkey (Sekisui XenoTech, Kansas City, KS) were incubated with 10 μM AT-511. At predetermined time points (0, 2, 4, 8, and 24 h post the start of incubation), hepatocytes were harvested, rinsed and extracted for AT-5010 prior to LC-MS/MS analysis.

LC-MS/MS analysis
- Plasma concentrations of AT-511 and its L-alanyl metabolite AT-551, nucleoside metabolite AT-273, and tissue levels of the triphosphate active metabolite AT-5010 were analyzed using validated LC-MS/MS methodologies. The lower limits of quantitation were 6 ng/ml for AT-511, AT-551 and AT-273 in plasma and 12 ng/g for AT-5010 in tissue samples, respectively.

Data analysis
- Plasma concentrations of AT-511, AT-551 and AT-273 were subjected to non-compartmental PK analysis using WinNonlin software (version 6.3 or above, Pharsight, Mountain View, CA).
- Clinical trial regimen was performed based on steady-state plasma PK data obtained from HCV-infected patients treated with 553 mg/d for AT-527 for 7 days (2), using MonolixSuite 2019 (Lixosoft, Antony, France).

Results

The parent produg (AT-511) is converted to the L-alanyl intermediate AT-551 which further undergoes multiple activation to the intracellular triphosphate active metabolite AT-5010, the predominant phosphate.

- AT-273 can only be formed via dephosphorylation of its intracellular phosphates, and therefore plasma AT-273 serves as the surrogate marker for intracellular AT-5010.

- After oral administration, plasma AT-511 was rapidly converted to AT-551 with gradual and sustained AT-273.
- At 12 h post the last dose (steady-state trough level), AT-273 plasma concentration was 0.13 ± 0.04 μM.

- At 12 h post the last dose in NHP, AT-5010 concentrations in lung and kidney were similar and higher than liver.
- The half-life (t½) of AT-5010 in lung (9.4 h) and kidney (8.0 h), approximately 2-fold longer than liver (4.3 h).
- Prediction of human tissue levels based on an observed ratio of 7 in human versus NHP concentrations of AT-5010 (Fig 3) as assessed by in vitro formation in primary hepatocytes suggested that lung intracellular levels of AT-5010 (at trough (12 h) will exceed 0.5 μM, the in vitro EC_{50} of AT-511 against SARS-CoV-2 replication in human airway epithelial (HAE) cell cultures.
- Predicted AT-9010 levels in human kidney and liver also exceed the in vitro EC_{50} of the drug.

Conclusions

- Simulations were performed for various regimens including QD and BID without or with a loading dose.
- Results indicate that a simple 550 mg BID regimen can rapidly achieve and consistently maintain throughout therapy lung levels of the active triphosphate metabolite AT-9010 exceeding the in vitro EC_{50} of AT-511 in inhibiting replication of SARS-CoV-2 in HAE cells.
- The predicted trough level of AT-9010 based on simulation was approximately 0.9 μM (Fig 4), which is in close agreement with the predicted trough of 0.98 μM (Table 1) based on in vivo tissue distribution data in NHP.

Fig. 1. Putative pathway for metabolism of AT-527 to its active triphosphate, AT-9010

Table 1. Actual (NHP) and predicted (human) tissue levels of AT-9010

<table>
<thead>
<tr>
<th>Species</th>
<th>Intracellular AT-9010 conc. at 12 h post dose (μM)</th>
</tr>
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<tbody>
<tr>
<td>NHP</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.089</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.13</td>
</tr>
<tr>
<td>Lung</td>
<td>0.14</td>
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</tbody>
</table>

| NHP           | Human  | 0.62 | 0.91 | 0.98 |

<table>
<thead>
<tr>
<th>Species</th>
<th>AT-511</th>
<th>AT-551</th>
<th>AT-273</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Concentration (μM)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
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References