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Metabolite Identification of Nucleoside Analog Prodrugs in Biological Matrix by Derivatization Coupled with Radio-Detector and Mass Spectrometry

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PURPOSE

Prodrugs of nucleoside analog are widely designed for the treatment of antiviral infections and anticancer chemotherapy. Metabolite profiling and identification is of utmost importance to understand the pharmacokinetics characteristics of the prodrugs. However, this task is often very challenging due to their strong polarity of the metabolites, especially for the mono-/di-/tri-phosphate metabolites, and endogenous interference from matrix. In addition, some metabolites are endogenous substances, hard to find and distinguish. Using radio-labelled compound as a tracer has a significant advantage of detecting the metabolites from endogenous matrix.

OBJECTIVE

This poster, provides a method of acetylation derivatization coupled with radioactivity determination to identify the metabolites of nucleoside analog prodrugs in biological matrix.

METHOD(S)

- **Samples:**

^{14}C-labeled DF-006 (adenosine monophosphate derivatives, labelled at adenine moiety), an ALPK1 agonist, was orally dosed to mice (20 mg / 100 µCi/kg). Mouse plasma (0.5, 2, 6, 24 h), urine (0-48 h), feces (0-48 h), and bile (0-48 h) samples were collected.

- **Metabolite profiling and characterization:**

Radiolabeled metabolite profiles of mouse plasma, urine, bile , and feces samples were determined using LC coupled with on-line or off-line radioactivity monitor (LC-RAM). The structures of detected metabolites were characterized by LC coupled with Q Exactive high-resolution mass spectrometry (LC-HRMS).

- **Acetylation:**

1 mL 0-24 h urine sample was dried under nitrogen stream. 50 µL of pyridine and 400 µL of acetic anyhydride were added, after vial-sealing, the vial was heated at 50°C for 4 h. The reaction was terminated by adding methanol followed by drying. The sample was reconstituted and submitted to LC-RAM/HRMS system to obtain radio-profiles and HRMS data.

RESULT(S)

A major metabolite, designated as M1, was detected in the radio-profiles of dosed urine samples. An ion of m/z 157.0360 at the retention time of the radio-peak M1 was observed in MS spectrum, which potentially corresponds to the [M-H]- ion peak of allantoin (Figure 1). Meanwhile, the ion peak was also detectable in mouse blank urine samples with similar ion intensity (Figure 2).

To investigate further, an acetylation derivatization was conducted for dosed urine samples. After acetylation, the original radio-peak M1 in dosed urine samples disappeared. Instead, one major and three minor radio-peaks (D1~D4) were observed. The ion peak of m/z 241.0578 at the retention time of the radio-peak D1 was proposed to be the [M-H]- ion peak of acetylation product of allantoin (allantoin + 2 COCH2) , and the ion peaks of m/z 265.0578 at the retention time of the radio-peak D2~D4 were proposed to be the [M-H]- ion peaks of acetylation products of allantoin (allantoin + 3 COCH2 – H2O) (Figure 3). Therefore, M1 was identified as allantoin.

Allantoin was also the major metabolite in plasma, bile and feces. (Data not shown)

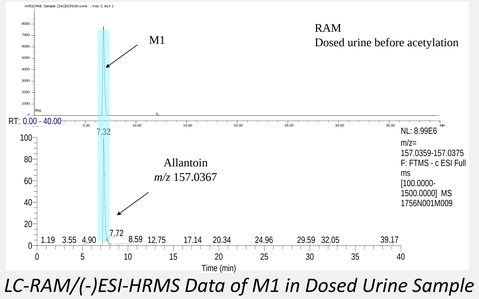

Figure 1. *LC-RAM/(-)ESI-HRMS Data of M1 in Dosed Urine Sample before Acetylation.*

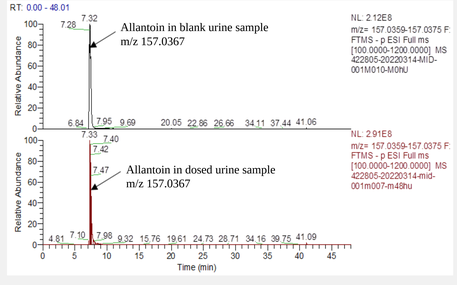

Figure 2. *LC-(-)ESI-HRMS Data of Allantoin in Blank and Dosed Urine Samples.*

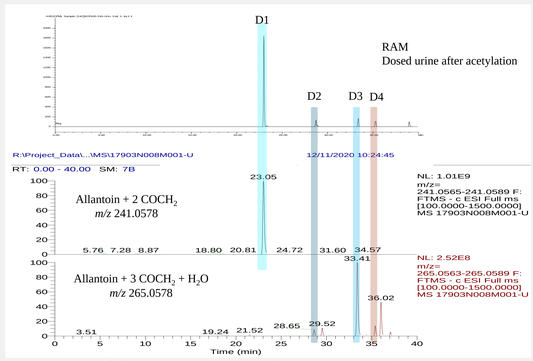

Figure 3. *LC-RAM/(-)ESI-HRMS Data of Derivatives of M1 in Dosed Urine Sample after Acetylation.*

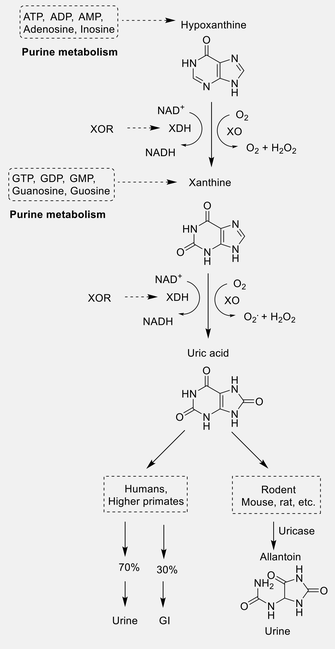

Figure 4. *Metabolic Pathways of Purine Metabolism in Animals and Humans.*

CONCLUSION(S)

Adenosine monophosphate derivatives may undergo a purine metabolic pathway, leading to the formation of xanthine, uric acid, and allantoin, which can be detected by MS both in dosed samples and blank samples, posing challenges in metabolite profiling and identification (Figure 4). Derivatization coupled with radioactivity determination proved to be a useful/valuable method for the identification of the metabolites, such as purine, pyrimidine and their analogs, particularly in the presence of endogenous matrix interference or when the metabolites have poor retention in HPLC systems.

ACKNOWLEDGMENTS

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REFERENCE

[1] C. Chen, *et al. Medical Science Monitor*. 2016, 22: 2501-2512.