Metabolite profiling and identification in dog plasma and tissues of edasalonexent (CAT-1004), a conjugate of docosahexaenoic acid (DHA) and salicylic acid (SA)

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OBJECTIVE
Edasalonexent (CAT-1004) is an orally administered small molecule candidate drug which inhibits NF-κB, a transcription factor driving inflammation, fibrosis and muscle degeneration that is activated in Duchenne Muscular Dystrophy (DMD). Edasalonexent is a conjugate of salicylic acid (SA) and docosahexaenoic acid (DHA) using the SM ART (Safely Metabolized And Rationally Targeted) LinkerSM drug discovery platform. This technology allows the simultaneous intracellular delivery of two bioactives to elicit a pharmacological response that can not be replicated by administering the bioactives separately or in combination. Edasalonexent is currently in clinical development in 4- to 7-year-old boys with DMD regardless of mutation.

The work described here was conducted to identify edasalonexent metabolites formed in beagle dogs in vivo and to determine their presence in plasma, heart, liver and skeletal muscle using liquid chromatography-high resolution mass spectrometry (LC-HRMS).

EXPERIMENTAL DESIGN
• Drug treatment: Beagle dogs were treated with 1000 mg/kg/day Edasa for 39 consecutive weeks. No treatment related signs of toxicity were recorded.
• Sampling: last day of administration after 39 weeks of treatment.
• Sample preparation:
  - For each matrix, samples were separately pooled per gender, time point and organ.
  - Plasma: protein precipitation (methanol + 0.1% formic acid).
  - Organs: homogenization and liquid-to-liquid extraction (acetonitrile/methanol (3/1 v/v) containing 0.1% of formic acid) followed by centrifugation.
• Metabolite ID:
  - Analysis: UHPLC -MS – data dependent - MS/MS acquisition mode using a Thermo Scientific Q Exactive Orbitrap (positive and negative ionization).
  - Data mining: expected metabolites (previous in vitro/in vivo studies), in silico metabolite prediction (MetWorks), precursor ions analysis.

RESULTS
Edasalonexent was bio-transformed into 13 metabolites in male dogs and 10 metabolites in females with large overlaps between the two genders.
• Among the tissues analyzed (plasma, heart, liver and skeletal muscle), liver showed the greatest number and highest levels of edasalonexent metabolites.
• In heart, liver and skeletal muscle of both male and female dogs, DHA (M51) represented between 80 and 99% of the total drug related material detected (as relative LC/MS peak area) containing 0.1% of formic acid) followed by centrifugation.
• Metabolite ID:
  - Analysis: UHPLC -MS – data dependent - MS/MS acquisition mode using a Thermo Scientific Q Exactive Orbitrap (positive and negative ionization).
  - Data mining: expected metabolites (previous in vitro/in vivo studies), in silico metabolite prediction (MetWorks), precursor ions analysis.

CONCLUSION
• In beagle dogs, CAT-1004 was metabolized by oxidation, glucuronidation, hydrolysis and shortening of the alkyl chain. In total 10 (females) to 13 (males) metabolites were formed. Not surprisingly the liver was the tissue with the largest number of detectable metabolites.
• There was a large overlap between the metabolic pathways of CAT-1004 in male and female dogs. Three male-specific (minor) metabolites and two female-specific metabolites were found.
• M51 (DHA) was present in greater quantities (from 5 to 10 fold) in tissues of treated animals compared with control animals. M51 was the principal CAT-1004 metabolite found in heart, liver and muscle of both genders, being largely more abundant than CAT-1004 itself.
• These results suggest that after administration of edasalonexent to beagle dogs DHA is preferentially retained by heart and skeletal muscle, two affected tissues in DMD disease, more than the parent compound and/or any other metabolite detected in the same tissues.
• DHA was also one of the major circulating metabolites observed in dogs.

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