Pharmacokinetic effects of Melanocortin Ligands in a ruminant model

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Introduction

- Multi-drug resistant organisms pose one of the most significant challenges to veterinary and public health
- Current antibiotics are unable to completely eradicate certain bacterial infections
- The lack of FDA approved antibiotics highlights the need for a new class of antibiotics
- Melanocortins are members of the cationic-aromatic class of antimicrobial peptides and as such, maintain various forms of antimicrobial activity
- One ligand developed by Tensive controls was TCMCB07
- B07, and various analogs have broad spectrum effects, including bactericidal effects on Gram positive and negative bacteria
- There is evidence that AMPs are effective against several infections, including those caused by several resistant species.
- The current study examined the pharmacokinetic profile of TCMCB07, a putative antimicrobial agent and analogue of innate immunity antimicrobial melanocortin peptides in a ruminant model.

Methodology

B07 Administration: Healthy Holstein bull calves (n = 6) eight weeks of age were used for this experiment. Physical examinations were performed initially to evaluate the health of the animal. The calves were randomly split into three-groups of two calves. Calves in group 1 (n = 2), were given a 300mg/15ml dose of anti-microbial peptide (AMP) subcutaneously. Group 2 (n = 2) received a 300mg/15ml dose of AMP IM followed by a 30mg dose IV after the initial sampling period of 24 hours. Group 3 (n = 2) received a 300mg/15ml dose per os (PO) with a worming gun.

Sample Collection: For each calf, blood samples (two ml) were collected by jugular venipuncture into EDTA-coated tubes before treatment (0 h) and at 1, 5, 10, 15, and 30 minutes immediately post-treatment. Additional samples were collected again at 1, 2, 4, 6, 8, 12 and 24 h post administration.

Sample Preparation. Samples were thawed at 25ºC. 200 µl of each plasma sample was precipitated with 800 µl acetonitrile. Samples were vortexed 10 sec and pelleted at 16 x g for 3 min in a microfuge. Supernatants were transferred to new tubes and dried in a rotary evaporator. Samples were resuspended in 500µl H_2O for High Performance Liquid Chromatography analysis (RP-HPLC).

Chromatography. RP-HPLC analyses were performed using a Gilson 321 H1 pump connected to a Panorama Fluorat-02 spectrofluorometer (Lumex Ltd.) with excitation set at 229 nm and fluorescence detection at 337 nm. Fluorescence was recorded using the Panorama 222-S software on a Gateway SX2855 computer. Injection was performed manually with a Rheodyne 7725i injector with a 1 ml sample loop (Rheodyne). Separation of analytes was achieved by gradient elution on an Agilent Pursuit C18 (5µm) column (250 mm x 4.6 mm I.D.) protected by a guard column (10 x 3.0 mm, ThermoFisher Scientific). Solvent A consisted of 0.1 HCl in H_2O and solvent B was 100% acetonitrile.

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Results

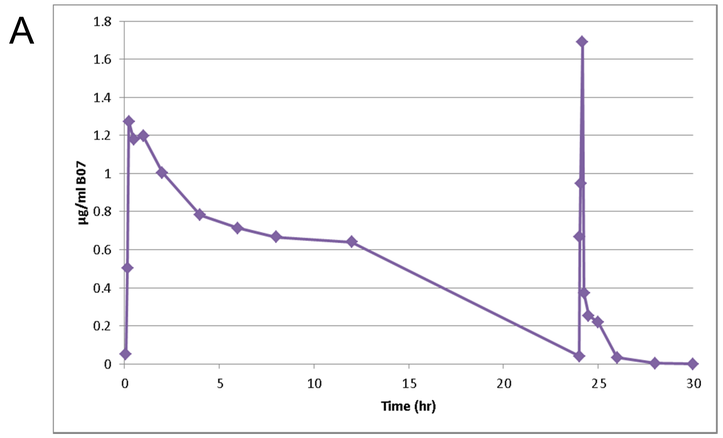

Fig 1. (A) Plasma B07 levels following IM injection of 300mg/15ml B07 solution with 24 hr sampling period, followed by IV injection with 24 hr sampling period. (B) Plasma B07 levels following SQ injection of 300mg/15ml B07 solution with 24 hr sampling period, and IV injection with 24 hr sampling period. (C) Plasma B07 levels following oral administration of 900mg/30ml B07 solution with 24 hr sampling period.

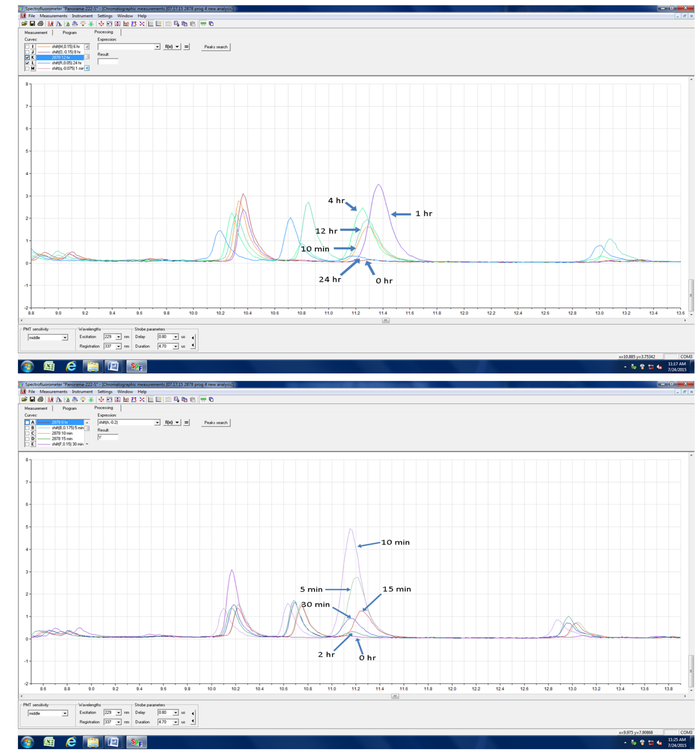

Fig 2. Representative chromatograms following IM (A) and IV (B) injections showing metabolite peaks, and B07 (labeled) peaks at various intervals.

Conclusions

Our focus was to identify TCMCB07 in steer plasma, and analyze the quantities detected following oral, subcutaneous, and intramuscular administration. From the preliminary results we were able to identify B07 in each sample. Following IM administration of a 300mg/15ml dose, we saw a peak in plasma B07 levels of 1.27 µg/ml at 15 minutes post-treatment. Subcutaneous administration, of the same dose, yielded a maximum B07 concentration of 2.19 µg/ml at 30 minutes post-treatment. Administering the same dose orally resulted in no B07 detected, while a 900mg/30ml dose administered orally, resulted in B07 detection at 7.5 hours post-treatment, with a slight increase to 0.11 µg/ml at 24 hours. Intravenous administration, following SQ and IM testing, led to a large spike in plasma B07 within 10 minutes of injection. Results from other samples are still pending.

Future Studies

- Once the pharmacokinetic properties of the AMP are determined, in the ruminant model, studies will then be conducted to determine the pharmacodynamics of these compounds in vivo
- These experiments will examine their antibacterial action in food animals, particularly ruminants.
- Preliminary pharmacokinetic measures will offer useful insights into therapeutic potential of AMP in ruminants.

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PP2	Please acknowledge the source of funding that you included in your submitted abstract here Pithua, Patrick, 7/27/2015
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