INS-3001 efficiently inhibits severe vascular calcifications by direct interference with vessel wall calcification

Anja VERHULST1, Mattias E. IVARSSON2, Roberto MAI2, Ellen NEVEN1, Patrick C. D’HAESE 1

Background and Aim
Prevention/treatment of vascular media calcification currently is based on controlling its most important risk factors. A new therapeutic approach with potential higher efficacy consists in the administration of molecules directly interfering with the calcification process in the medial layer of the vessel wall, such as INS-3001. This poster reports about the administration of INS-3001 in a rat model of vitamin D-warfarin induced vascular media calcifications (pooled data of 3 independent studies).

Materials and Methods
- Calcification was induced in male rats (8 weeks) by warfarin (3 mg/kg diet) administration during 5 days and 4 consecutive daily administrations of vitD3 (100.000 IU/kg) starting on day 1 of the warfarin administration.
- Rats were randomly assigned to different groups: vehicle, and INS-3001 groups of 1x12.5, 1x25, 2x25, 1x50 and 2x50 mg/kg/day.
- Treatment was administered sc. during 7 days.
- Animals were sacrificed after 7 days.
- Vascular calcification was evaluated on Von Kossa-stained tissue sections of the thoracic/abdominal aorta and by measurement of the total Ca content of the thoracic/abdominal aorta and the carotid/femoral arteries by atomic absorption spectrometry.
- Calcaemic status was checked for 30 min after the first treatment administration in the vehicle and the INS-3001 1x50 mg/kg/day group, by measuring ionized calcium (i-STAT point of care analysis).
- In the figure below a schematic overview of the study set-up is presented.

Results
Mortality rate was 38% (15/40) in the vehicle group, 42% (5/12) in the 1x12.5 mg/kg dose group, 33% (4/12) in the 1x25 mg/kg dose group, 21% (4/19) in the 1x50 mg/kg dose group, 15% (2/13) in the 2x25 mg/kg dose group and 0% (0/18) in the 2x50 mg/kg dose group. Kaplan Meier survival analysis found the mortality rate in the latter group to be significantly different from the vehicle group (p = 0.04).

Calcification in the medial layer of the thoracic aorta was significantly lower on Von Kossa stained sections (area% positivity) of the INS-3001 treated groups compared to the vehicle group (16±5%, 8±5%, 6±4%, 15±1%, 20±3% and 28±10% in respectively the 2x50, 1x50, 2x25, 1x25, 1x12.5 mg/kg/day and the vehicle group). Total Ca content of the thoracic aorta was also significantly lower in the INS-3001 (not the 12.5 and 25 mg/kg/day) groups compared to the vehicle group (2.6±3.9, 7.4±6.9, 6.1±5.0, 14.9±7.0, 15.6±9.4 and 14.9±7.5 mg/g tissue in respectively the 2x50, 1x50, 2x25, 1x25, 1x12.5 mg/kg/day and the vehicle group).

Calcification in the medial layer of the abdominal aorta was significantly lower on Von Kossa stained sections (area% positivity) of the INS-3001 treated groups compared to the vehicle group (3±5%, 8±7%, 6±4%, 15±1%, 20±3% and 28±10% in respectively the 2x50, 1x50, 2x25, 1x25, 1x12.5 mg/kg/day and the vehicle group). Total Ca content of the abdominal aorta was also significantly lower in the INS-3001 (not the 12.5 and 25 mg/kg/day) groups compared to the vehicle group (2.8±3.7, 7.4±6.9, 6.1±5.0, 14.9±7.0, 15.6±9.4 and 14.9±7.5 mg/g tissue in respectively the 2x50, 1x50, 2x25, 1x25, 1x12.5 mg/kg/day and the vehicle group).

INS-3001 administration did not influence the calcaemic status of the animals. Serum levels of ionized calcium were similar after vehicle and INS-3001 (50 mg/kg) administration: respectively 5.5±0.2 mg/dl and 5.4±0.2 mg/dl 30 minutes post treatment (Cmax INS-3001).

Conclusion
INS-3001 is a promising molecule for the treatment of vascular calcifications. The inhibitory effect is expected to be independent of the underlying cause of calcifications.

Contact: anja.verhulst@uantwerpen.be