SNF472 PHARMACOKINETICS-PHARMACODYNAMICS IN A VITAMIN D-INDUCED CALCIFICATION RAT MODEL

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INTRODUCTION

SNF472, an intravenous (i.v.) formulation of phytate or myo-inositol hexaphosphate (IP_6), exhibits beneficial properties in calcium related diseases such as the prevention of renal lithiasis[1], osteoporosis[2] and cardiovascular calcification (CVC)[3] by binding and blocking the growing sites of the hydroxyapatite (HAP) crystal. This formulation is currently being developed as a treatment for CVC in hemodialysis patients.

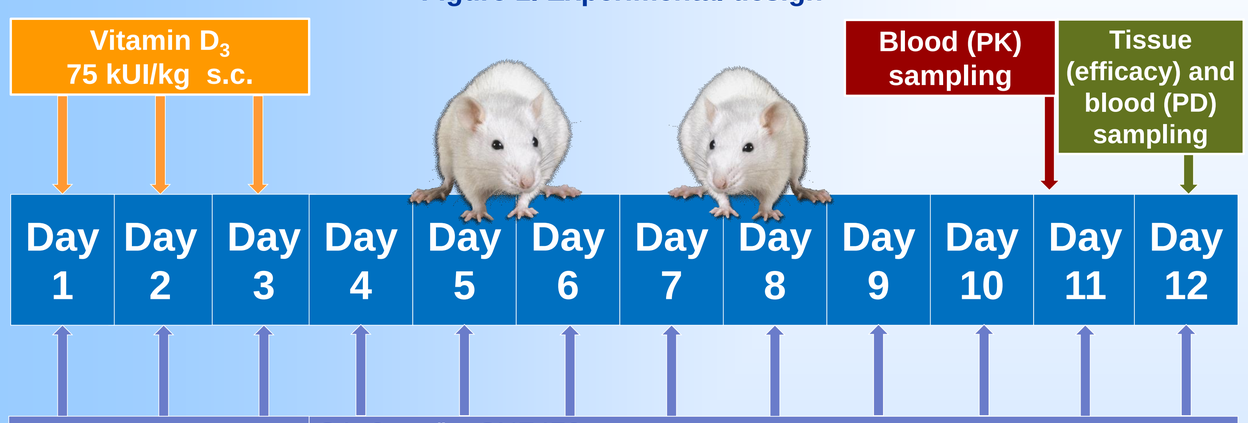
AIM

To evaluate the efficacy and the pharmacokinetics-pharmacodynamics (PK/PD) relationship of the i.v. infusion of SNF472 in a vitamin D rat model.

MATERIALS AND METHODS

A total of 40 Sprague Dawley rats were divided into four groups of nine to ten animals. Calcification was induced by subcutaneous (s.c.) administration of 75,000 IU/kg Vitamin D for three consecutive days (day 1 to day 3). Treatment or saline were daily administered for 12 days by 4-hour i.v. infusion starting on day 1. Groups 1, 2 and 3 received 3, 10 and 30 mg/kg SNF472, respectively; group 4 received saline. Animals were sacrificed on day 12. The experimental design is summarized in Figure 1. **Efficacy:** Aorta and heart were collected to determine calcium content by inductively coupled plasma optical emission spectrometry (ICP-OES) after acidic digestion as a measure of CVC. **PK:** Blood was collected on day 11 to evaluate SNF472 plasma levels before infusion, and 60 and 240 minutes after the infusion start. SNF472 levels were quantified by UPLC-MS[4]. **PD:** Blood was collected at the end of the last infusion immediately before sacrifice to measure the effect of SNF472 on the ex vivo blood crystallization potential (PD assay). HAP crystallization was induced ex vivo by the addition of 12.5 mM Ca^{2+} and 1.5 mM HPO_4^{2-} to plasma samples and followed by spectrophotometric measurements at 550 nm for 30 minutes.

Figure 1. Experimental design

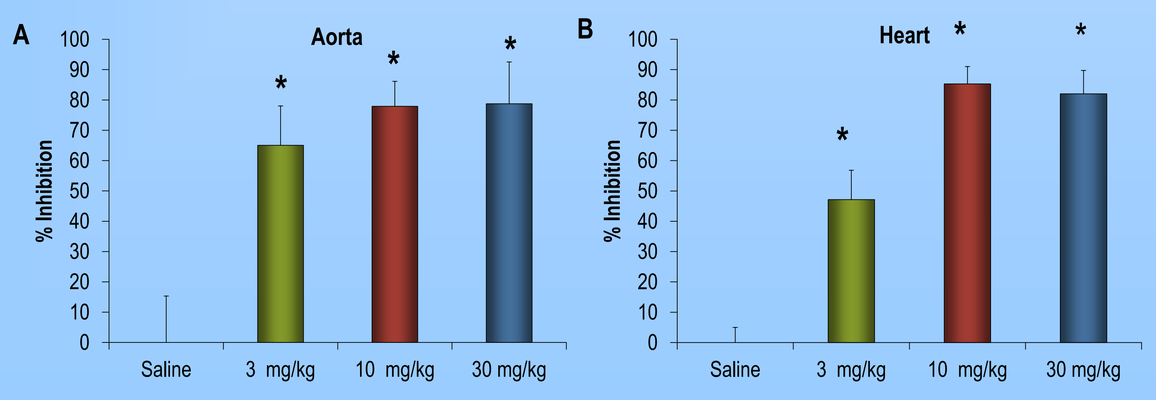


Treatment 4 h i.v. infusion	
	G1: 3 mg/kg SNF472
	G2: 10 mg/kg SNF472
	G3: 30 mg/kg SNF472
	G4: Saline

RESULTS

Efficacy: CVC was inhibited by SNF472 (Figure 2). The 3 mg/kg dose was able to inhibit calcification up to 65 and 50% in aorta and heart, respectively. Maximum efficacy (80%) in both tissues was achieved with the dose of 10 mg/kg and maintained at 30 mg/kg. The IC_{50} values were calculated for aorta and heart as 0.5 and 3.1 µg/mL (0.8 and 4.6 µM) of SNF472, respectively.

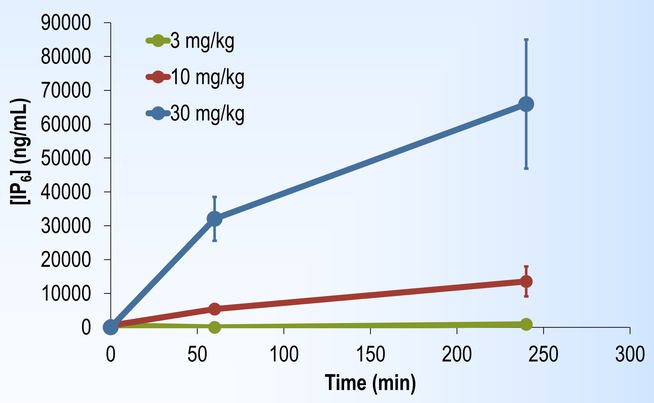
Figure 2. Inhibition of (A) aorta and (B) heart calcification in rats treated with 4 hours intravenous infusion of SNF472 after calcification induction with 3 x 75 kIU/kg vitamin D



Results represent mean ± S.E.M. Statistical analysis: One-way ANOVA. (*) Differences vs Saline, $p < 0.05$

PK: IP_6 plasma levels were undetectable (LLOQ of 0.5 µg/mL) for the first hour of infusion in the group treated with 3 mg/kg, but quantifiable levels were attained at 4 hours. SNF472 levels were quantifiable at 1 hour in the groups treated with 10 and 30 mg/kg and lineal to the administered dose. C_{max} of 0.9, 13.5 and 65.9 µg/mL (1.3, 20.5 and 99.9 µM) were achieved at 4 hours after dosing 3, 10 and 30 mg/kg of SNF472, respectively. These results are shown in Figure 3 and Table 1.

Figure 3. Pharmacokinetics of SNF472 in calcifying rats treated with 3 x 75 kIU/kg vitamin D



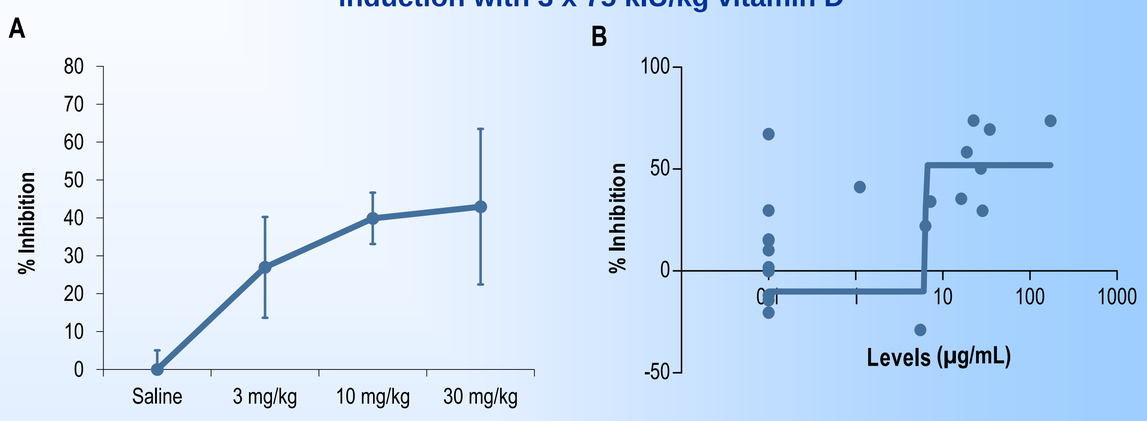
Results represent mean ± S.E.M.

Group	C_{max} (µg/mL)
3 mg/kg	0.9 ± 0.2
10 mg/Kg	13.5 ± 4.4
30 mg/Kg	65.9 ± 19.0

Table 1. C_{max} of IP_6 after SNF472 administration in calcifying rats treated with 3 x 75 kIU/kg vitamin D.
Results represent mean ± S.E.M.

PD: Treatment with increasing doses of SNF472 inhibited the ex-vivo formation of calcium crystals in plasma with a clear dose-response (Figure 4A). The inhibition of crystallization potential in plasma was also related to IP_6 plasma levels at C_{max}. As observed in Figure 4B, a relation could be obtained and an IC_{50} of 6.3 µg/mL (9.6 µM) was calculated. Considering a 70% of IP_6 protein binding, the IC_{50} for free IP_6 would be 1.9 µg/mL (2.9 µM). Therefore, a direct correlation exists between the reduction of CVC and the PD assay results.

Figure 4. (A) Pharmacodynamic assay of inhibition of plasma crystallization potential and (B) relation between IP_6 circulating levels and inhibition of plasma crystallization potential in rats treated with 4 hours intravenous infusion of SNF472 after calcification induction with 3 x 75 kIU/kg vitamin D



(A) Results represent mean ± SEM of all animals analyzed per group. (B) Individual data, results represent mean ± S.E.M. of 6 replicates per sample

CONCLUSIONS

1. **SNF472 i.v. infusion dose-dependently inhibits CVC in rats induced by vitamin D reaching a maximum of 80% inhibition with doses of 10 mg/kg or higher.**
2. **A PK/PD relationship is observed as the presence of SNF472 in plasma in the micromolar range decreases plasma crystallization potential and correlates with CVC inhibition.**
3. **PK/PD measurements predict CVC inhibition and the PD assay is suggested as a valuable tool to evaluate the effect of CVC inhibitors in clinical trials with hemodialysis patients.**

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This study was supported by a RETOS COLABORACIÓN: RTC-2014-2460-1 ISCIII grant (Ministerio de Economía y Competitividad. Government of Spain)

Cofinanciado por el Fondo Europeo de Desarrollo Regional (FEDER). Unión Europea. Una manera de hacer Europa