INTRAVENOUS SNF472 INHIBITS VITAMIN D INDUCED CARDIOVASCULAR CALCIFICATION IN RATS

J. Perelló[1,2], C. Salcedo[1], M. Ketteler[3], F. Tur[2], E. Tur[1], B. Isern[1], P.H. Joubert[1], M.D. Ferrer[1]

[1] Laboratoris Sanifit SL. Research and Development Department, 07121 Palma de Mallorca, Spain
[2] Laboratory of Renal Lithiasis Research, IUNICS, University of the Balearic Islands, 07122 Palma de Mallorca, Spain
[3] Division of Nephrology, Klinikum Coburg GmbH, 33, D-96450 Coburg, Germany
Contact: joan.perello@sanifit.com

KIDNEYWEEK 2014
Philadelphia, PA | Nov 11 - 16

INTRODUCTION

Cardiovascular calcification has been shown to be an independent predictor of cardiovascular events in CKD patients. SNF472 under development by SANIFIT, is an intravenous formulation of myo-inositol hexaphosphate or phytate, a small and highly water-soluble molecule that inhibits calcification by binding to the growing sites of the hydroxyapatite (HAP) crystal. Beneficial properties have been attributed to this compound in calcium related diseases such as the prevention of renal lithiasis[1], osteoporosis[2], cardiovascular calcification[3], sialolithiasis[4] and dental tartar[5].

AIM

(1) To investigate *in vivo* the effects of SNF472, an intravenous (i.v.) formulation of phytate, on vitamin D induced vascular calcification, and (2) to evaluate *in vitro* the effect of SNF472 on hydroxyapatite (HAP) binding kinetics.

MATERIALS AND METHODS

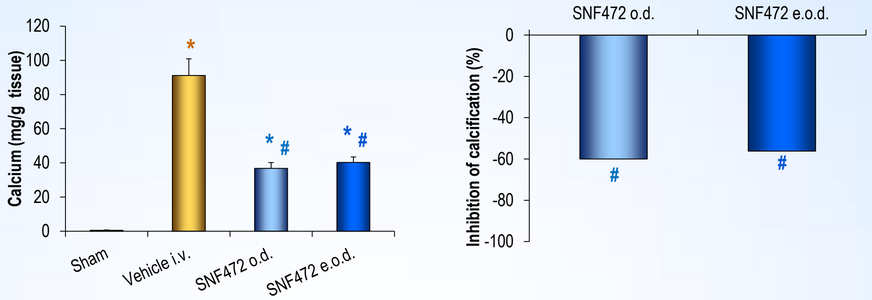
1. Four groups of 5-7 male Sprague Dawley rats (total of 26) were studied. A control group received i.v. vehicle daily. Two treated groups received 1 mg/kg SNF472 i.v. either daily (o.d) or every other day (e.o.d). A sham group was used as a non-calcified control. Calcification was induced by 5 daily oral administrations of 75 kIU/kg of vitamin D_3 starting on day 3 of treatment. Rats were sacrificed on day 14 and aortas and hearts were removed and digested in HNO_3:$HClO_4$ (1:1) to quantify total calcium by ICP-OES. Calcium and phosphorus content was additionally determined in serum by ICP-OES.

2. SNF472 binding kinetics on HAP was studied *in vitro* by incubation of 130 mg HAP in the presence of 5000 ng/mL SNF472 at 37 ºC and pH 7.40 for up to 8 hours. SNF472 release from HAP was studied by incubating pre-bound SNF472-HAP in fresh 0.05 M Tris buffer, pH 7.40, at 37 ºC for up to 3 days. SNF472 was determined through quantification of total phosphorus by ICP-OES in the HAP samples eluted through a chromatographic column prepared with anion exchange resin to separate SNF472 from inorganic phosphate.

RESULTS

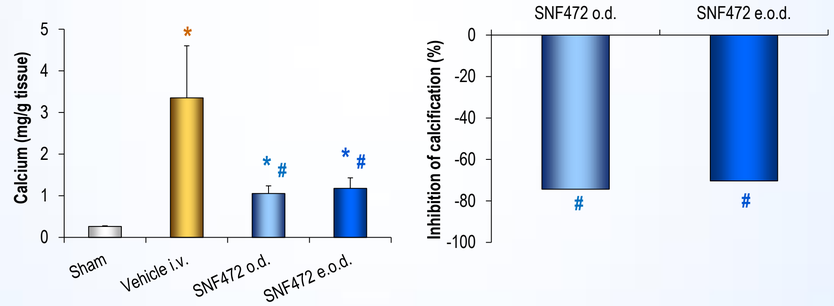
The administration of vitamin D_3 induced a marked increase in aortic and heart calcium levels. Both o.d and e.o.d. i.v. administration of SN472 at 1 mg/kg resulted in reductions of calcification by 55-60% in aorta and 70-75% in heart.

Figure 1. Aorta calcification induction by 5 consecutive daily p.o. administrations of 75,000 IU/kg vitamin D_3 and inhibition of calcification by 14 daily (o.d.) or 7 every other day (e.o.d.) 1 mg/kg i.v. administration of SNF472



Statistical analysis: One-way ANOVA. (*) Differences vs sham, (#) differences vs vehicle, $p < 0.05$

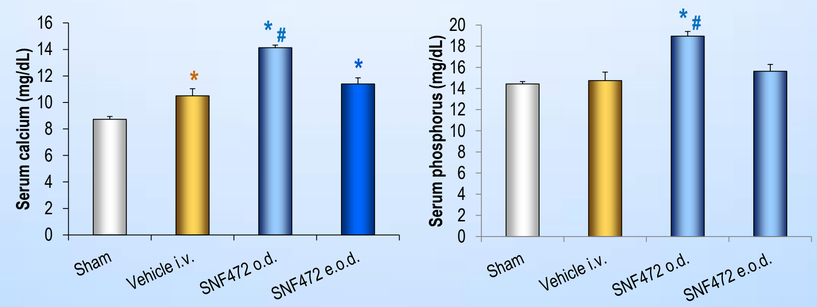
Figure 2. Heart calcification induction by 5 consecutive daily p.o. administrations of 75,000 IU/kg vitamin D_3 and inhibition of calcification by 14 daily (o.d.) or 7 every other day (e.o.d.) 1 mg/kg i.v. administration of SNF472



Statistical analysis: One-way ANOVA. (*) Differences vs sham, (#) differences vs vehicle, $p < 0.05$

Serum calcium concentration increased in all vitamin D treated groups compared to the sham group, and was significantly increased by daily SNF472 treatment. Higher circulating phosphorus levels were also evidenced in the rats treated with daily SNF472.

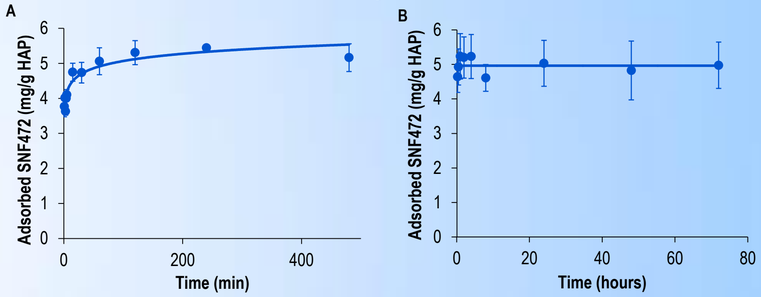
Figure 3. Calcium and phosphorus serum levels in rats treated with i.v. 1 mg/kg SNF472 for 14 days



Statistical analysis: One-way ANOVA. (*) Differences vs sham, (#) differences vs vehicle, $p < 0.05$

SNF472 was bound *in vitro* to hydroxyapatite crystals almost immediately, reaching an 80% of maximum adsorption after 5 minutes of incubation, and maximum adsorption (about 5 mg/g) at 60 minutes. This high affinity of SNF472 for HAP was confirmed after studying its release from the crystal surfaces, as no sign of release was evidenced after 3 days of incubation in fresh, non-SNF472-containing buffer.

Figure 4. (A) Binding and (B) release kinetics of SNF472 on hydroxyapatite crystals.



These results are compatible with the efficacy found *in vivo* when administered e.o.d. and point to a possible use of SNF472 for the prevention of cardiovascular calcification in ESRD patients and calcification-related pathologies such as calciphylaxis.

CONCLUSIONS

Both o.d. and e.o.d. i.v. administration of 1 mg/kg SNF472 inhibit up to 60 and 70% calcification progression in aorta and heart, respectively.

E.o.d. administration is as effective as o.d. administration but additionally does not increase calcium serum levels, a side effect observed when the compound was administered daily.

These results point to a possible use of SNF472 in the treatment of cardiovascular calcification in ESRD patients and calcification-related disorders such as calciphylaxis.

BIBLIOGRAPHY

(1) Scand J Urol Nephrol 2000; 34:162-164 **(2)** Front Biosci 2010;1:1093-1098
(3) Front Biosci 2006;11:136-142 **(4)** Clin Chim Acta 2003;334:131-136
(5) J Periodontal Res 2009;44:616-621