INTRAVENOUS SNF472 INHIBITS VITAMIN D INDUCED CARDIOVASCULAR CALCIFICATION IN RATS

J. Perelló1,2, C. Salcedo1, M. Ketteler3, F. Tur1, E. Tur1, B. Isern1, P.H. Joubert1, M.D. Ferrer1

Laboratories Sanfit 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@sanfit.com

INTRODUCTION

Cardiovascular calcification has been shown to be an independent predictor of cardiovascular events in CKD patients. SNF472 under development by SANFIT, 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 lithiasis1, osteoporosis2, cardiovascular calcification3, sialolithiasis4 and dental tartar5.

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 kl/kg of vitamin D3 starting on day 3 of treatment. Rats were sacrificed on day 14 and aortas and hearts were removed and digested in HNO2:HClO4 (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 D3 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 D3 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

Figure 2. Heart calcification induction by 5 consecutive daily p.o. administrations of 75,000 IU/kg vitamin D3 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

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

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.

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