SNF472 INHIBITS THE PROGRESSION OF VITAMIN D INDUCED CARDIOVASCULAR CALCIFICATION IN RATS

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INTRODUCTION

Cardiovascular calcification (CVC) has been shown to be an independent predictor of cardiovascular events in CKD patients. SNF472 under development by SANIFIT is a formulation of myo-inositol hexaphosphate, 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], CVC[3], sialolithiasis[4] and dental tartar[5].

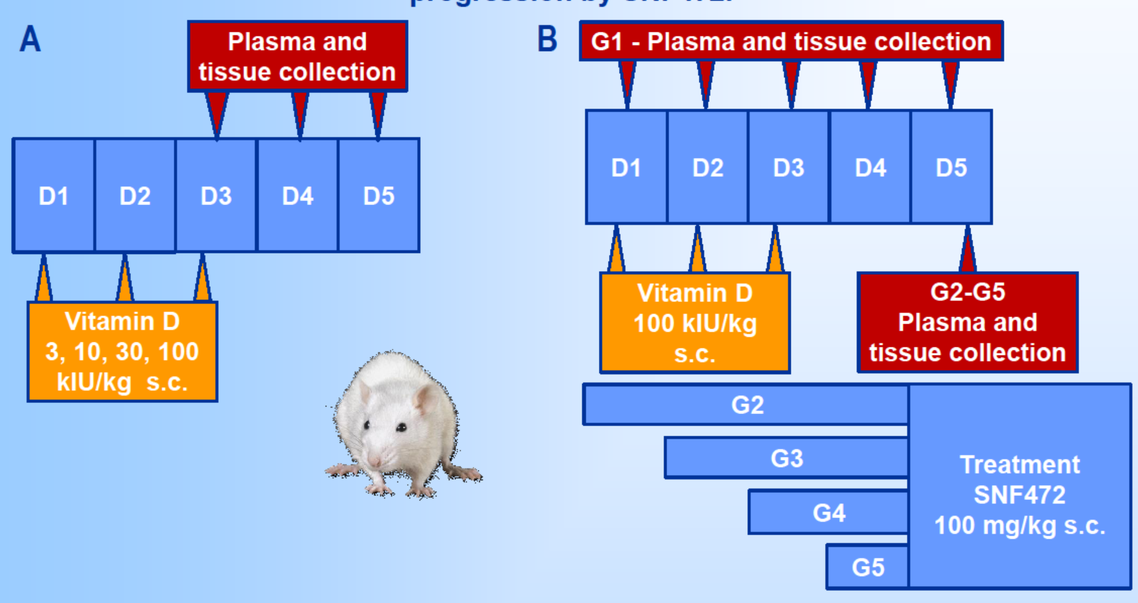
AIM

To investigate the effects of SNF472, a formulation of phytate, on vitamin D induced CVC progression, after setting up a new model for the rapid induction of CVC with subcutaneous (s.c.) vitamin D_3 in rats.

MATERIALS AND METHODS

A total of 87 male Sprague Dawley rats were used in this study. Of these 45 were used to evaluate the dose-response of vitamin D in producing CVC. Calcification was induced through daily (day 1 to day 3) s.c. administration of 3, 10, 30, 100 or 300 kIU/kg of vitamin D_3. A subset of three animals from each group was sacrificed and evaluated on days 3, 4 and 5 (Figure 1A). The remaining 42 animals were administered 100 kIU/kg vitamin D and divided into five groups, each group receiving daily s.c. vehicle or SNF472 for up to five days (Figure 1B). G1 received daily vehicle; three animals were sacrificed every day from day 1 to day 4 to evaluate CVC progression and 6 animals were sacrificed on day 5. Four more groups of six animals (G2, G3, G4, G5) were dosed with SNF472 at 100 mg/kg, starting on day 1, 2, 3 or 4, respectively, and sacrificed on day 5.

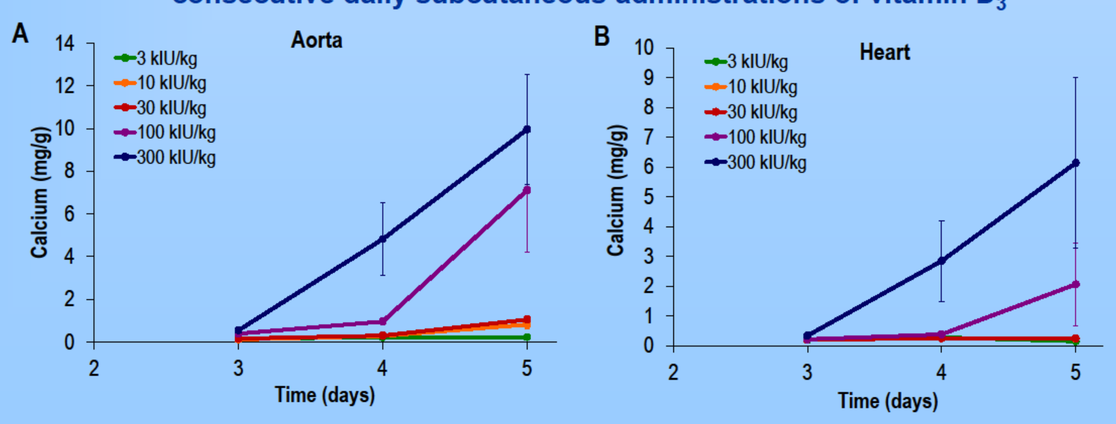
Figure 1. Experimental design. (A) Dose-response and progression of cardiovascular calcification induced by vitamin D_3. (B) Inhibition of cardiovascular calcification progression by SNF472.



RESULTS

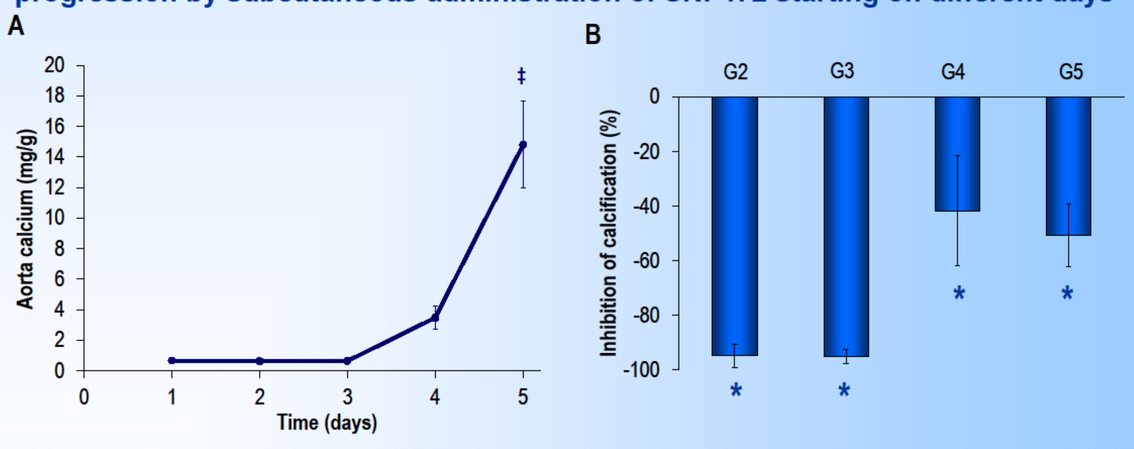
CVC was induced in rats when vitamin D was administered at doses above 30 kIU/kg. The time course of calcification was similar in aorta and heart, where calcification became evident during the fourth day of study and continued increasing by day 5, as shown in Figure 2.

Figure 2. Time course induction of (A) aorta and (B) heart calcification by three consecutive daily subcutaneous administrations of vitamin D_3



The same profile of calcification induction was obtained in the second experiment in the control group that did not receive SNF472, as seen in Figure 3A. There was a relationship between dose-regimen and the response relationship to SNF472 (Figure 3B). G2 receiving four doses of SNF472 showed a reduction of aorta calcification progression of 95%. G5 receiving a single dose on day 4 showed a reduction of calcification progression of 51%. The groups in between had intermediate effects.

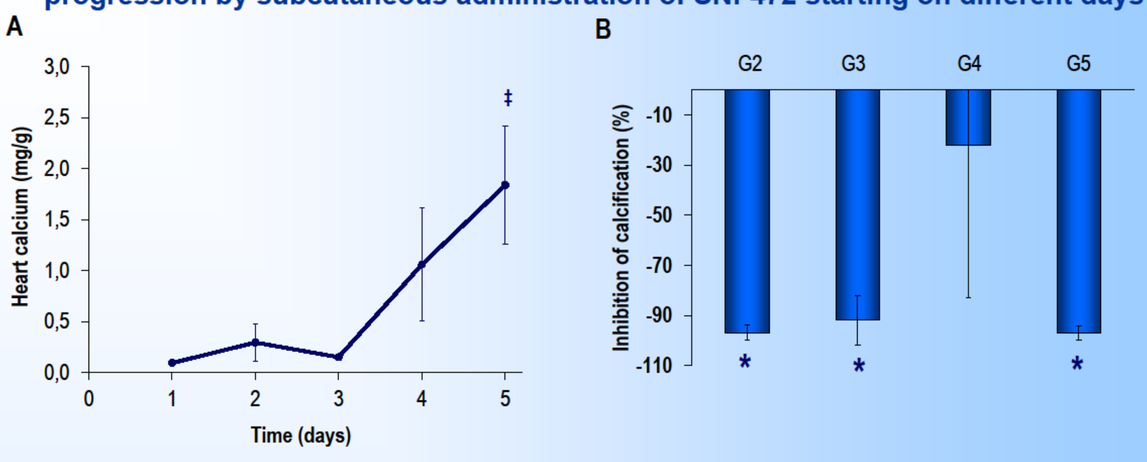
Figure 3. (A) Progression of aorta calcification after three consecutive daily subcutaneous administrations of 100 kIU/kg vitamin D_3. (B) inhibition of calcification progression by subcutaneous administration of SNF472 starting on different days



G2 received four daily 100 mg/kg SNF472 doses, starting on D1. G3 received three daily 100 mg/kg SNF472 doses, starting on D2. G4 received two daily 100 mg/kg SNF472 doses, starting on D3. G5 received one 100 mg/kg SNF472 dose on D4. Statistical analysis: One-way ANOVA. (‡) Differences vs. day 1; (*) Differences vs control, $p < 0.05$

Similar results were obtained in heart, with inhibition of calcification progression between 90 and 100%, independently of the day of administration, with the one exception of G4 receiving two doses of SNF472 in which a lower than expected efficacy was found. These results are shown in Figure 4.

Figure 4. (A) Progression of heart calcification after three consecutive daily subcutaneous administrations of 100 kIU/kg vitamin D_3. (B) inhibition of calcification progression by subcutaneous administration of SNF472 starting on different days



G2 received four daily 100 mg/kg SNF472 doses, starting on D1. G3 received three daily 100 mg/kg SNF472 doses, starting on D2. G4 received two daily 100 mg/kg SNF472 doses, starting on D3. G5 received one 100 mg/kg SNF472 dose on D4. Statistical analysis: One-way ANOVA. (‡) Differences vs. day 1; (*) Differences vs control, $p < 0.05$

These results evidence that even dosing the compound for one day, where a franc and significant calcification is already produced in all animals, the efficacy of SNF472 on the reduction of calcification progression is 50% in aorta and 100% in heart, suggesting that SNF472 is able to work at different steps of the calcification process, being active even at the strongest conditions in this model, where the HAP crystal has been already formed and deposited in the tissue.

CONCLUSIONS

1. **SNF472 is able to slow-down calcification progression induced by vitamin D in a rat model.**
2. **SNF472 is effective in preventing calcification induction but also in inhibiting ongoing calcification progression.**
3. **These results suggest that SNF472 might be an alternative therapeutic principle for cardiovascular calcification treatment.**

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