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CONCISE REPORT

The H2 blocker famotidine suppresses progression of ossification of the posterior longitudinal ligament in a mouse model

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ABSTRACT

Background: Ossification of the posterior longitudinal ligament (OPLL) of the spine is a common human myelopathy that leads to spinal cord compression. No disease-modifying drug for OPLL has been identified, whereas surgery and conservative management have been established.

Objectives: To evaluate the therapeutic potential of the H2 blocker famotidine for ectopic ossification in the cervical spine in an OPLL mouse model.

Methods: The H2 blocker famotidine was orally administered to *Enpp1*$^{ttw/ttw}$ mice, a model of OPLL, at either 4 or 15 weeks of age. Radiological and survival rate analyses were performed to assess the effects of famotidine on OPLL-like lesions and mortality in *Enpp1*$^{ttw/ttw}$ mice.

Results: Oral administration of famotidine suppressed the progression of OPLL-like ectopic ossification and reduced mortality in *Enpp1*$^{ttw/ttw}$ mice when administration began at 4 weeks of age, early in the development of ossification.

Conclusions: This study points to the use of famotidine as a disease-modifying drug for ectopic ossification of spinal soft tissue, including OPLL.

Key messages

- Only a few candidates for disease-modifying drugs for OPLL have been proposed.
- Oral administration of famotidine suppressed the progression of OPLL-like ectopic ossification in a mouse model.
- The finding may reposition H2 blockers, which has been widely used as gastrointestinal agents, for the treatment of intractable OPLL.

INTRODUCTION

Ossification of the posterior longitudinal ligament (OPLL) of the spine is a common human myelopathy.[1 2] The ossification progresses slowly, but leads to spinal cord compression. OPLL is a multifactorial disease caused by genetic as well as environmental factors, although a number of susceptibility genes has been reported.[3] Conservative management is preferred for patients without myelopathy, but surgery is usually necessary for neurological symptoms.[4] There is a relatively high incidence of surgical complications in cervical OPLL compared with that in other cervical degenerative diseases. Complete removal of ossified foci is difficult and leads to postoperative progression and recurrent neurological symptoms.[5] Although disease-modifying drugs for OPLL might prevent this, only a few candidates, including bisphosphonate and a P2 purinoceptor Y1 (P2Y1) antagonist, have been proposed.[6] There is a need to develop drugs targeting the progression of OPLL.

Cimetidine, a histamine receptor H2 (Hrh2) antagonist (H2 blocker), was reported to improve shoulder calcific tendinitis symptoms.[7] We recently identified the inhibitory effect of another H2 blocker, famotidine, on osteogenic differentiation of tendon cells in vitro.[8] Oral administration of famotidine also decreased the calcified region in the Achilles tendon of *Enpp1*$^{ttw/ttw}$ mice,[8] which carried a point mutation for the ectonucleotide pyrophosphatase/phosphodiesterase 1 (*Enpp1*) gene.[9] *Enpp1* is a susceptibility gene for OPLL,[3 10] and *Enpp1*$^{ttw/ttw}$ mice have been proposed as a model for OPLL.[9] Based on these factors, we hypothesised that H2 blockers might negatively affect progression of OPLL as well as tendon calcification.

In this study, we aimed to evaluate the therapeutic potential of famotidine for ectopic ossification in the cervical spine in the *Enpp1*$^{ttw/ttw}$ OPLL model mouse line.

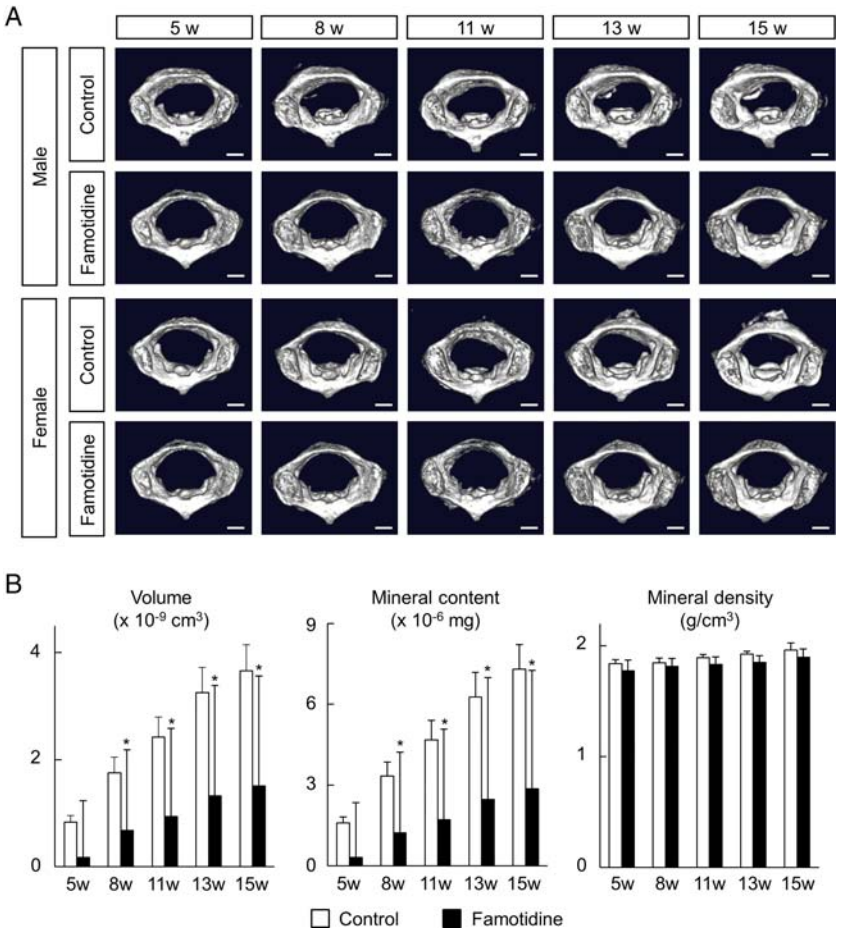

Figure 1 Radiological findings on the development of ossification of the posterior longitudinal ligament (OPLL)-like ectopic ossification in famotidine-treated *Enpp1*$^{ttw/ttw}$ mice. (A) Representative micro-CT images of cervical spines of *Enpp1*$^{ttw/ttw}$ mice treated with or without famotidine. Famotidine was orally administered from 4 weeks of age. Micro-CT was performed at the indicated weeks of age. Bars=1 mm. (B) Quantitative analyses of OPLL-like ectopic ossification in *Enpp1*$^{ttw/ttw}$ mice treated with or without famotidine from 4 weeks of age. Four male and female mice were analysed in each group (n=8). X-axes indicate ages of mice. *p<0.05.

METHODS

Details are in online supplementary methods. Famotidine was orally administered to *Enpp1*$^{ttw/ttw}$ mice at either 4 or 15 weeks of age at a dose of 0.667 µg/g/day. Ectopic ossification around the cervical spine was quantitatively analysed using sequential micro-CT. Quantitative data were expressed as mean±SD; statistical significance was evaluated using analysis of variance and Student t test. All experiments were performed in accordance with the protocol approved by the Animal Care and Use Committee of The University of Tokyo (#KA12-5).

RESULTS

Progression of ectopic ossification in cervical spines is suppressed by famotidine administration

To evaluate the effects of H2 blockers on OPLL-like ectopic ossification, famotidine was administered orally to *Enpp1*$^{ttw/ttw}$ mice. Since ossification becomes evident around 8 weeks of age,[11] oral administration of famotidine was started at 4 weeks of age. Each group consisted of four male and four female mice. At 5, 8, 11, 13 and 15 weeks of age, ectopically ossified regions in cervical spines were quantified using reconstructed three-dimensional micro-CT images.

All 16 *Enpp1*$^{ttw/ttw}$ mice tested exhibited ectopic ossification of the cruciform ligament in the atlanto-occipital area by 8 weeks of age, as previously reported[11]; the extent of ossification increased throughout the observation period (figures 1A,B and 2). The ectopic ossification was smaller in the famotidine group than in controls (figure 1A). Quantitative analyses revealed that volume and mineral content of calcified ligaments were both significantly smaller in the famotidine group than in controls (figure 1B), but mineral density was not significantly different. Figure 2 shows individual variability of quantitative data in each group; female mice tended to have more severe ectopic ossification than male mice (see online supplementary figure S1).

To gain insights into potential adverse effects of famotidine on bone metabolism, we measured serum calcium levels at 1, 3, 6 and 24 h in WT mice after single-shot famotidine administration (see online supplementary figure S2A), and serum calcium levels and bone mass in *Enpp1*$^{ttw/ttw}$ mice exposed to 1-month administration (see online supplementary figure S2B and S2C). Neither calcium levels nor bone mass were largely changed by famotidine administration compared with the relevant controls (see online supplementary figure S2A–C).

Survival rates are improved by famotidine in *Enpp1*$^{ttw/ttw}$ mice

We assessed survival rates in *Enpp1*$^{ttw/ttw}$ mice with or without famotidine. To examine the effect of famotidine

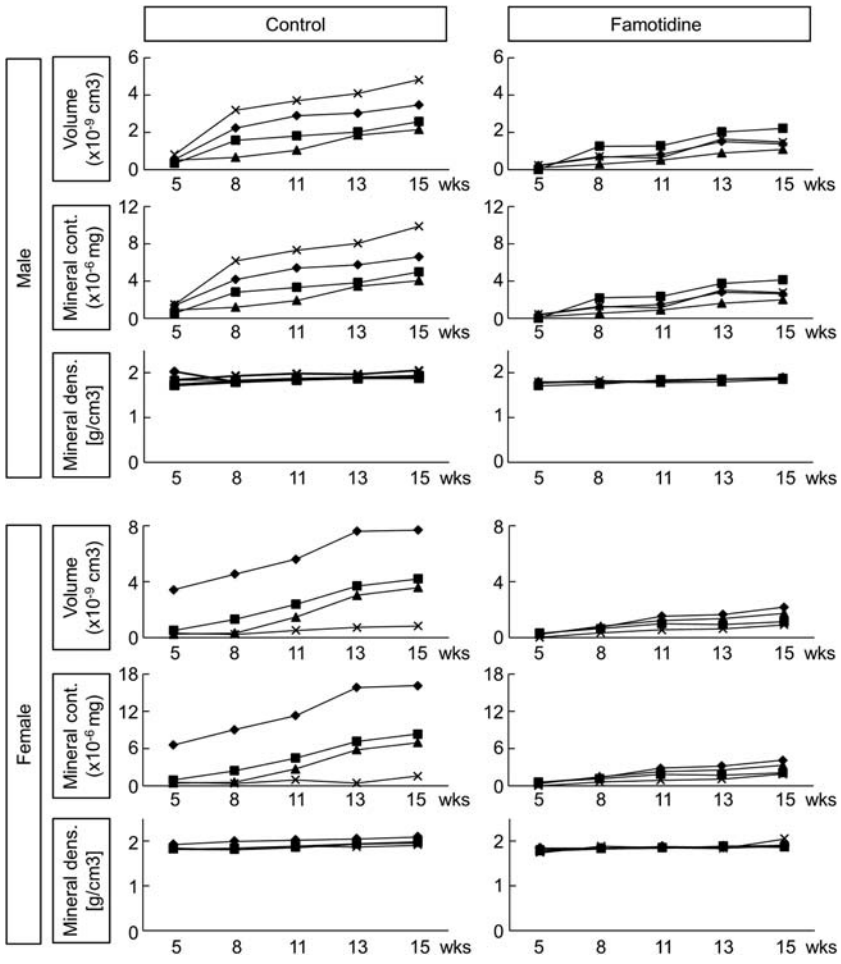

Figure 2 Change over time in ossification of the posterior longitudinal ligament (OPLL)-like ectopic ossification in individual *Enpp1*$^{ttw/ttw}$ mice reported in figure 1. Quantitation of OPLL-like ectopic ossification for each *Enpp1*$^{ttw/ttw}$ mouse either treated with famotidine from 4 weeks of age or controls. X-axes indicate ages of mice. Mineral cont., mineral content; Mineral dens., mineral density.

on more advanced ectopic ossification in cervical spines, we created another treatment group, with famotidine administered from 15 weeks of age. Thus, we analysed *Enpp1*$^{ttw/ttw}$ mice treated with famotidine from 4 weeks of age (5 males and 8 females), those treated from 15 weeks of age (5 males and 7 females), and controls that received no famotidine (3 males and 8 females). Figure 3 shows that mice exposed to famotidine from 4 weeks of age lived longer than those exposed to famotidine from 15 weeks of age or controls. There was no marked difference in survival rates between the latter two groups. Female mice died earlier than male mice. These data suggest that famotidine administration from an early phase of the disease progression can reduce mortality caused by ectopic ossification in the cervical spine, but exhibits little effect on more advanced disease.

DISCUSSION

Our study results suggest that famotidine can act as a disease-modifying drug for ectopic ossification of spinal soft tissue, potentially repositioning H2 blockers, widely used as gastrointestinal agents, for the treatment of intractable OPLL. We further propose that famotidine may be suitable for preventing recurrence after surgery for OPLL, but not for reversing established lesions, since delayed administration resulted in less improvement in the survival rate in *Enpp1*$^{ttw/ttw}$ mice. The gender difference in the reduction of mortality of *Enpp1*$^{ttw/ttw}$ mice by famotidine may be attributable to the more advanced ectopic ossification in females than in males. In addition, the distinct penetrance of *Enpp1*$^{ttw/ttw}$ phenotypes may underlie the lower survival rate in the group exposed to the delayed administration of famotidine compared with the control group.

Cellular and molecular mechanisms for H2 blockers on OPLL were not considered in this study. How does famotidine exert its therapeutic effects on ectopic ossification in the cervical spine? Histopathology of OPLL suggests that ectopic bone formation, in particular through endochondral ossification, mediates the disease;[12 13] degenerative changes in elastic fibres and cartilage formation were associated with OPLL,[12] and lesions had Haversian canals and marrow cavities.[13] Our previous data showed that famotidine suppresses osteoblast marker gene expression in the tendon cell line TT-D6.[8] H2 blockers may similarly negatively affect ectopic bone formation in spinal ligaments.

Besides *Enpp1*, two factors have been proposed in the pathogenesis of OPLL, based on in vivo data: runt-related transcription factor 2 (*Runx2*) and Indian hedgehog (*Ihh*). *Runx2*, a master regulator of osteogenesis,[14 15] is expressed in OPLL, and loss of one copy of

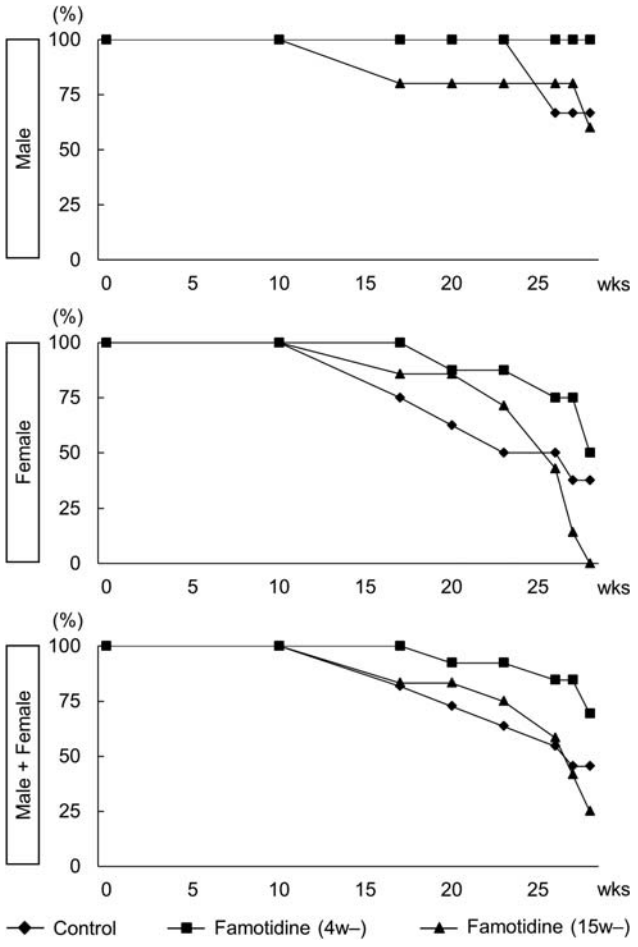

Figure 3 Survival rates of famotidine-treated *Enpp1*$^{ttw/ttw}$ mice. Survival rates were analysed in *Enpp1*$^{ttw/ttw}$ mice treated with famotidine from 4 weeks of age (■: 5 male and 8 female mice at the outset) and 15 weeks of age (▲: 5 male and 7 female mice at the outset) as well as controls (◆: 3 male and 8 female mice at the outset). X-axes indicate ages of mice.

Runx2 affects OPLL-like lesions under the *Enpp1*$^{ttw/ttw}$ background.[11] Sugita *et al*[16] demonstrated the expression of Ihh in both histological sections and primary cells from patients with OPLL. Ihh is required for osteoblastogenesis, and coordinates osteogenesis and chondrogenesis during endochondral ossification.[17 18] Recent genome-wide association studies identified additional OPLL-associated factors.[3 19] Hrh2 signalling may thus crosstalk with these pathways, underlying the suppressive action of H2 blockers on OPLL development. It is also possible that H2 blockers act on OPLL in an indirect manner through cells other than those in spinal ligaments. We are therefore now investigating Hrh2 signalling between the OPLL-related molecules above, which will be reported on in the near future.

In order to apply the present findings to a clinical setting, we need to consider the adverse effects of H2 blockers on bone mass and/or serum calcium levels, given that the involvement of histamine signalling in bone homoeostasis has been reported with a particular focus on osteoclastogenesis.[20] Although the results of our limited number of analyses suggest that such adverse effects are unlikely, further large-scale studies will be necessary to verify both the adverse and therapeutic effects on OPLL.

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