

Ferric citrate hydrate (JTT-751), a new phosphate binder, inhibits phosphorus absorption and prevents the progression of ectopic calcification, secondary hyperparathyroidism, and bone abnormalities in chronic renal failure rats.

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Supplementary Table 1 Criteria for Grading Aortic Lesions (histopathological examination)

Findings	Criteria for Grading
Mineralization	<p>In HE-stained sections, findings on calcification (mineralization), i.e. deposition of basophilic granular substances in vascular smooth muscle cells in the region from the intima to the media of the aortic wall and adjacent elastic fibers with degeneration of vascular smooth muscle cells and elastic fibers.</p> <ul style="list-style-type: none"> • Negative (-): No mineralization is observed. • Very slight (\pm): Deposition of basophilic granular substances in the aortic wall is unclear but seen to be localized. The percentage of the lesion in the entire aortic cross-section (section formed by cutting the aorta in round slices) is 5% or less. • Slight (+): Deposition of basophilic granular substances in the aortic wall is seen to be localized. The percentage of the lesion in the entire aortic cross-section (section formed by cutting the aorta in round slices) is 5% to 30%. • Moderate (2+): Deposition of basophilic granular substances in the aortic wall is seen clearly. The lesion occupies approximately one-half of the entire aortic cross-section (section formed by cutting the aorta in round slices) (30% to 70%). • Severe (3+): Deposition of basophilic granular substances in the aortic wall is seen clearly and is sometimes accompanied by localized tissue damage such as fragmentation of elastic lamellae. The lesion occupies almost all the entire aortic cross-section (section formed by cutting the aorta in round slices) (70% or more).
Von Kossa-positive cells	<p>In von Kossa-stained sections, findings on calcification (mineralization), i.e. formation of foci in which calcium salts that deposited on vascular smooth muscle cells in the region from the intima to the media of the aortic wall and adjacent elastic fibers are stained black to brown through the von Kossa reaction.</p> <ul style="list-style-type: none"> • Negative (-): No von Kossa-positive cells are found. • Very slight (\pm): Von Kossa-positive cells (black – brown) are seen to be localized. The percentage of the lesion in the entire aortic cross-section (section formed by cutting the aorta in round slices) is 5% or less. • Slight (+): Von Kossa-positive cells are seen to be localized. The percentage of the lesion in the entire aortic cross-section (section formed by cutting the aorta in round slices) is 5% to 30%. • Moderate (2+): Multifocal lesions containing von Kossa-positive cells are observed. The lesion occupies approximately one-half of the entire aortic cross-section (section formed by cutting the aorta in round slices) (30% to 70%). • Severe (3+): Multifocal to diffuse lesions containing von Kossa-positive cells are observed. The lesion occupies almost all the entire aortic cross-section (section formed by cutting the aorta in round slices) (70% or more).

Supplementary Table 2 Criteria for Grading Parathyroid Lesions (histopathological examination)

Findings	Criteria for Grading
Hyperplasia	<p>An increased number of cells (chief cells) composing the parathyroid gland. The parathyroid gland was enlarged in terms of its sectional area with the progression of hyperplasia, so as to push its surrounding tissue (thyroid gland). This is generally accompanied by cell hypertrophy (the cell nucleus is rounded and enlarged, and the cytoplasmic area is increased).</p> <ul style="list-style-type: none"> • Negative (-): No parathyroid hyperplasia is found. • Very slight (\pm): The parathyroid gland is slightly enlarged compared to that in an untreated control group but less than 1.5-fold larger than that in an untreated control group in area and it is unclear whether the enlarged parathyroid gland pushes its surrounding tissue or not. Localized cell hypertrophy is seen, or even when diffuse cell hypertrophy is seen, it is very slight. • Slight (+): The parathyroid gland is enlarged 1.5- to 2-fold above that in an untreated control group in area and is seen to push slightly the surrounding tissue. Slight but diffuse cell hypertrophy is also observed. • Moderate (2+): The parathyroid gland is enlarged 2- to 3-fold above that in an untreated control group in area and is seen to push moderately the surrounding tissue. Severe and diffuse cell hypertrophy is also observed. • Severe (3+): The parathyroid gland is enlarged more than 3-fold above that in an untreated control group in area and is seen to push severely the surrounding tissue. Severe and diffuse cell hypertrophy is also observed.

Supplementary Table 3 Criteria for Grading Femur Lesions (histopathological examination)

Findings	Criteria for Grading
Porosity	<p>Mainly, intracortical porosis. That is often accompanied by the appearance of osteoclasts. Very slight porosis may be seen to be localized even in normal animals according to the position where the tissue is sectioned and the state of bone metabolism equilibrium.</p> <ul style="list-style-type: none"> • Negative (-): Porosity is rarely observed. • Very slight (\pm): Localized intracortical porosis is seen in the cortical bone, or porosis is slight, even if it is diffuse. • Slight (+): Slight but diffuse intracortical porosis is seen. • Moderate (2+): Diffuse intracortical porosis is seen, but bone matrices are predominant over porous spaces. • Severe (3+): Diffuse intracortical porosis is seen and porous spaces are dominant over bone matrices.
Fibrosis	<p>Fibrous components increased in the cortical bone and endosteum, which was accompanied by proliferation of fibroblasts. Fibrosis is often increased with an increase in the degree of porosity. Even in normal animals, very slight fibrosis may occur, depending on the state of bone metabolism equilibration.</p> <ul style="list-style-type: none"> • Negative (-): Fibrosis is rarely observed. • Very slight (\pm): Localized fibrosis is found in the cortical bone and the endosteum of the spongy bone, or the lesion is slight, even if it is diffuse. • Slight (+): Slight fibrosis is found in the cortical bone and the endosteum of the spongy bone. • Moderate (2+): Fibrous lesions fill porous spaces, especially in the cortical bone. Diffuse fibrous lesions are also seen in the endosteum of the spongy bone, but bone matrices are predominant over fibrous lesions. • Severe (3+): Fibrous lesions fill porous spaces, especially in the cortical bone. Diffuse fibrous lesions are also seen in the endosteum of the spongy bone and fibrous lesions are predominant over bone matrices.
Osteoid formation	<p>Formation of uncalcified and immature bone matrices. These sections are stained homogeneously eosinophilic with HE. The osteoid is formed in a multilayer structure in the endosteum. The boundary between the osteoid and the bone matrix is relatively clear because of a difference in their staining properties.</p> <ul style="list-style-type: none"> • Negative (-): Osteoid formation is rarely observed. • Very slight (\pm): Localized osteoid formation is seen in the endosteum, or osteoid formation is slight, even if it is diffuse. • Slight (+): Localized to diffuse osteoid formation is found in the endosteum. • Moderate (2+): Diffuse osteoid formation is found in the endosteum. In some cases, osteoid matrix may look as if it protrudes in the medullary cavity due to the thickness of the osteoid seams, but there is no possibility that the medullary cavity is filled with osteoid tissues. • Severe (3+): Diffuse osteoid formation is found in the endosteum. The osteoid seams comparable in quantity to the thickness of the cortical bone are formed. This is remarkable, especially in the spongy bone, and the medullary cavity may be filled with a large quantity of osteoid tissues according to the site of the resection.

Supplementary Table 4. Effects of JTT-751 on body weight, diet consumption and serum mineral parameters in normal rats

<i>parameters</i>	Control	0.3% JTT-751	1% JTT-751	3% JTT-751	3% Calcium carbonate
<i>Body weight (g, Day8)</i>	346.5 ± 25.2	352.6 ± 15.4	348.7 ± 27.8	345.6 ± 24.5	348.0 ± 19.5
<i>Diet consumption (g/day)</i>	24.8 ± 2.1	26.2 ± 1.9	25.2 ± 2.4	24.5 ± 2.5	25.7 ± 1.6
<i>Serum intact PTH (pg/mL) (Day8)</i>	166.7 ± 26.6	164.8 ± 20.9	146.0 ± 27.0	110.2 ± 12.1 ‡	103.2 ± 9.3 ##
<i>Calcium (mg/dL) (Day8)</i>	10.40 ± 0.39	10.37 ± 0.53	10.62 ± 0.43	10.66 ± 0.11 §	11.05 ± 0.43 **
<i>1,25-(OH)₂D₃ (pmol/L) (Day8)</i>	822.6 ± 217.0	693.8 ± 221.8	832.3 ± 213.9	701.3 ± 209.7	613.0 ± 140.7 *

Data are mean ± SD. Control, n = 9; 0.3% JTT-751, n = 8; 1% JTT-751, n = 9; 3% JTT-751, n = 9; 3% Calcium carbonate, n = 9

‡p<0.01; ##p<0.05; §p<0.05; *,**p<0.05,0.01 vs. Control group.

Supplementary Table 5. *In vitro* binding ability of JTT-751 to phosphate

	Amount of phosphorus bound by JTT-751 (mg phosphorus/g JTT-751)
pH 2	74 ± 3
pH 2 to 7	46 ± 2
pH 7	4 ± 2

An aqueous phosphate solution (pH 2) and a JTT-751 solution (pH 2) were mixed and the pH was adjusted to 2. Similarly, an aqueous phosphate solution (pH 7) and a JT-751 solution (pH 7) were mixed and the pH was adjusted to 7 (the first reaction: concentration of JTT-751, 2.5 mg/mL; concentration of phosphate: 40 mmol/L). These were shaken at room temperature for 1 hour and then the pH was maintained (pH 2 or pH 7) or was changed from 2 to 7 (the latter reaction: concentration of JTT-751 2.0 mg/mL; concentration of phosphate 32 mmol/L). These were shaken at room temperature for further 1 hour, and following that, the reaction mixtures were filtered (0.22 µm, PVDF membrane, Millipore Corporation, Billerica, MA) to separate the deposited insoluble salts. The respective filtrates were used as the samples for the quantification of phosphorus. The phosphorus concentration of each filtrate was quantitated using a phosphorus measurement kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and the amount of phosphorus bound by JTT-751 (mg phosphorus/g JTT-751) was calculated based on the difference between the phosphorus concentrations in the presence and absence of JTT-751. The amounts of bound phosphorus (mg phosphorus/g JTT-751) under reaction conditions of pH 2, pH 2 to 7, and pH 7 were individually displayed as mean ± standard error.