Discovery of New Proteinase Inhibitor for the Treatment of Osteoporosis

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I. Bone Remodeling

II. Cathepsin K as a New Target for the Treatment of Osteoporosis

III. Discovery of Cathepsin K Inhibitors

Osteoporosis

"... a systemic skeletal disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures."

τ Normal

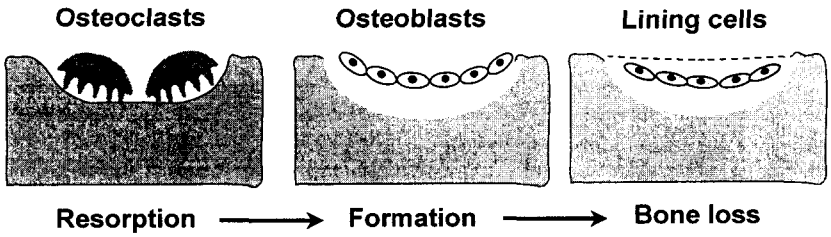
Abnormal υ

Major osteoporotic fractures

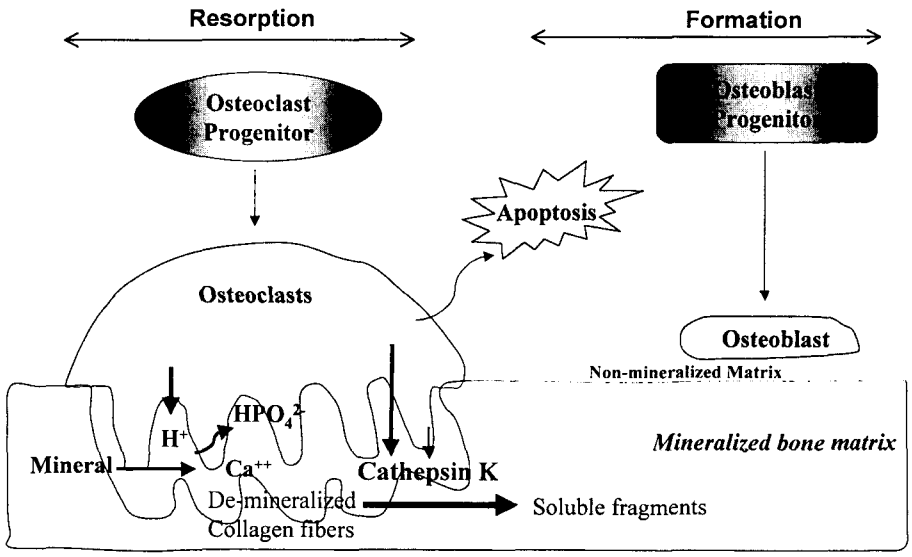
	Type of fractures		
	Colles'	Vertebral	Hip
Typical age (years)	>55	>65	>75
Women:Men	4:1	3:1	2:1
Predominant type of bone	Trabecular	Trabecular	Cortical

I. Bone Remodeling

Bone remodeling process is so extensive that it is completely regenerates the adult skeleton every ten years.(*Endocrine Rev.* 2000, 21:115)



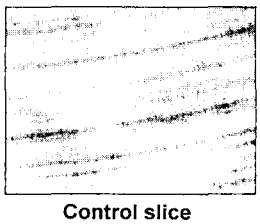
I-1. Mode of Action



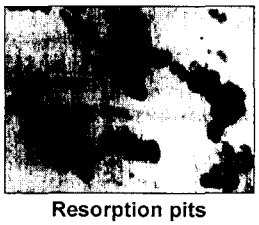
I-2. *In vitro* Bone Resorption Assay; Pit Assay

1. Methods

- culture of the disaggregated osteoclast
- cell isolation from neonatal rabbits
- dentine-slice based osteoclast resorption assay
- resorption measurement by direct quantification of pits or CTx

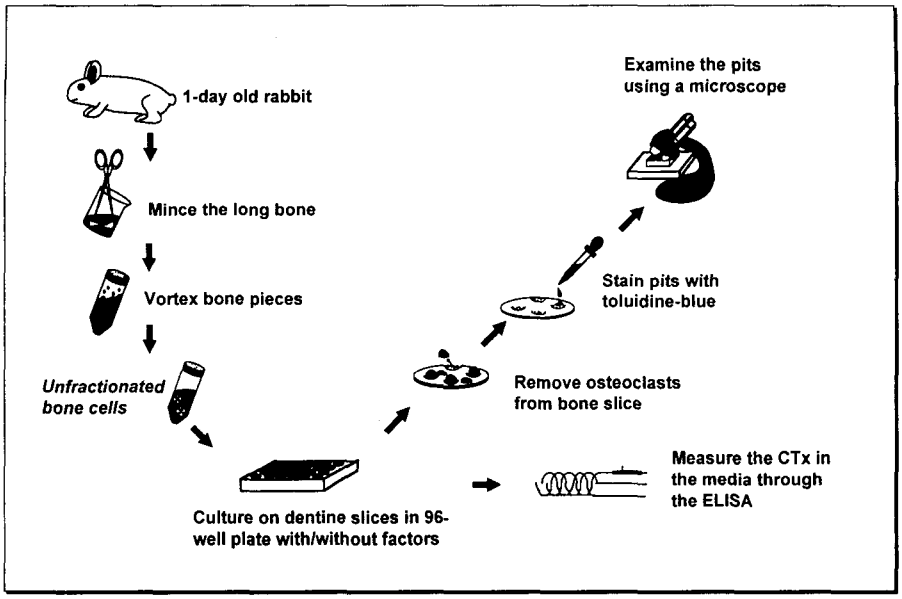


Control slice

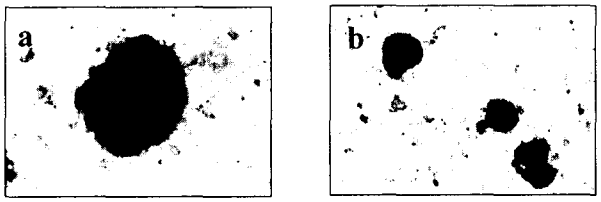


Resorption pits

2. Schematic view of pit assay

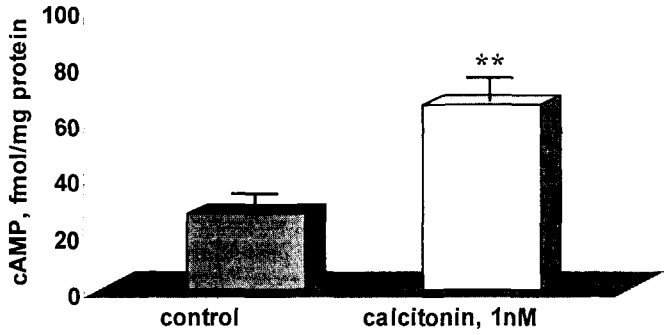


3. Identification of osteoclasts by TRAP staining

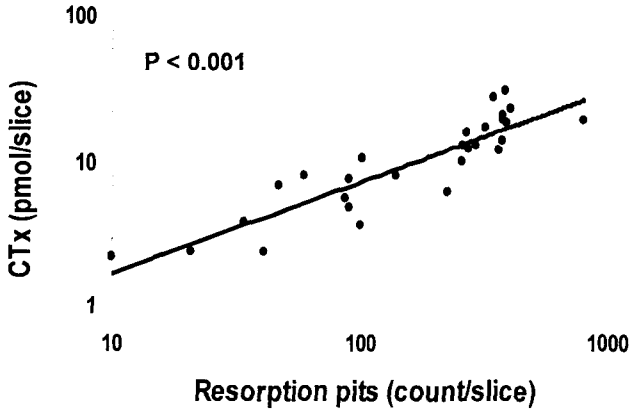


Part of isolated bone cells were cytospun for TRAP staining. Magnification 100x(a); 40x(b). TRAP-positive and multinucleated (over 3 nuclei) giant cells were counted as $TRAP^+$-osteoclasts and the frequency was always > 0.1%.

4. Cytosolic cAMP increase by calcitonin treatment on unfractionated rabbit osteoclasts

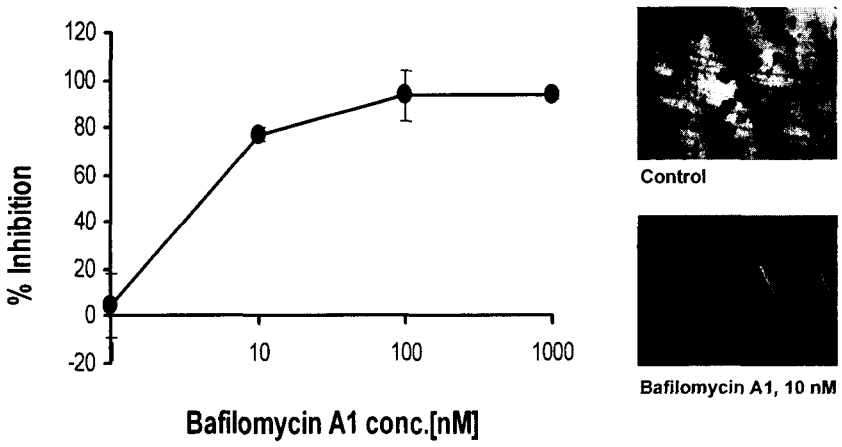


5. Correlation between CTx and pit counts



Unfractionated bone cells ($1x10^5$ ~ $1x10^6$ cells/well) were cultured on bovine femoral cortical bone slices for 3 days. The number of pits were counted under light microscope and CTx concentration was measured by ELISA.

6. Inhibition by bafilomycin A1 of osteoclast-mediated bone resorption



II. Cathepsin K as a New Target for the Treatment of Osteoporosis

1. Molecular biology of cathepsin K

- Location of gene: CTSK maps to 1q21 (Gelb B.D. et al., 1997).
- Protein: a 329-amino acid preprocathepsin K
- Homology: More than 50% identity to both cathepsin S and cathepsin L in propeptide sequence
- Expression of mRNA: Human breast cancer cell (BCC) lines BT-20, MCF-7, Hs578T, MDA-MB-231, SKBR3, ZR75-30, BT549, MDA-MB-468, T-47D [Littlewood-Evans A.J. *et al.*, 1997]

2. Cathepsin K inhibitor as a novel target

- Highly specific distribution in osteoclast
- Predominant cysteine protease in osteoclast
- Major role in proteolysis of bone matrices
- Similar substrate specificity shared with cathepsin S
- Most homologous with cathepsin L in a.a. sequence of mature enzyme

3. Tissue-limited distribution in human osteoclast

Expression of cathepsins mRNA in human bone, osteoclastoma (GCT), and a panel of human tissues by in situ hybridization

Tissue	Cat. B	Cat. S	Cat. L	Cat. K
Bone osteoclasts	Negative[a]	Negative	Negative[a]	+++
GCT osteoclasts	Negative[b]	Negative	Negative[b]	+++
Cartilage chondroclasts	Negative	Negative	Negative	+++
Spleen	+++[c]	ND[d]	++[c]	Negative
Liver	++[c]	ND	++[c]	Negative
Kidney	++[c]	ND	±[c]	Negative

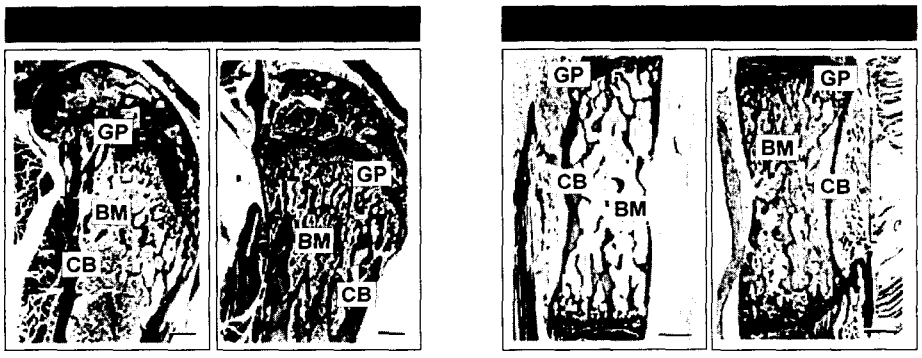
[*J Biol Chem* 1996 271(21):12511-6]

4. Predominant cysteine protease in osteoclast

Total lysosomal cysteine protease activity from rabbit osteoclast	100%
• Cathepsin K-like activity	60%
• Cathepsin L-like activity	10%
• The rest	30%

[*J Biochem (Tokyo)* 1998 Apr;123(4):752-9]

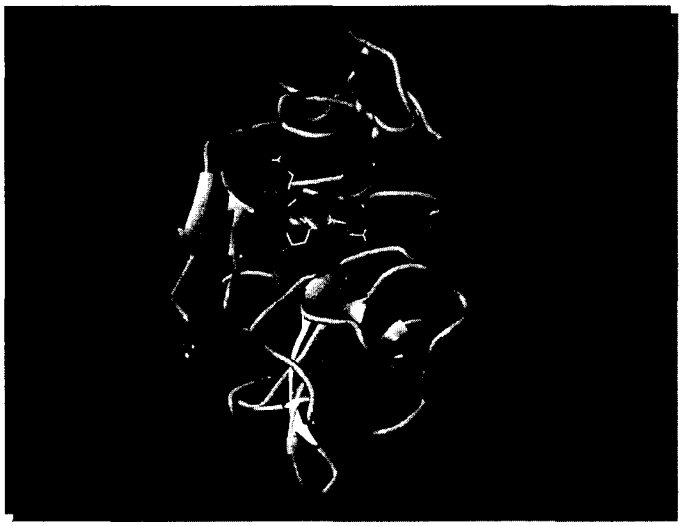
5. Osteopetrosis in Cathepsin K-deficient mice



Control mice | Cat.K-deficient mice | Control mice | Cat.K-deficient mice

BM, bone marrow; CB, cortical bone; GP, growth plate. [*PNAS*, 1998 (95)13453-8]

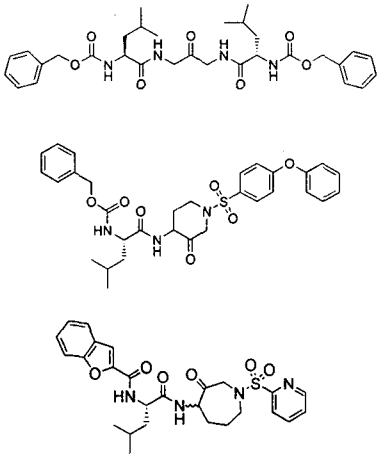
III. Discovery of Cathepsin K Inhibitors



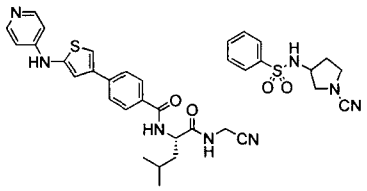
Discovery of the potent, selective and orally absorbable cathepsin K inhibitors which efficiently and specifically suppress the osteoclastic bone resorption *in vivo*

Structures of cathepsin K inhibitors

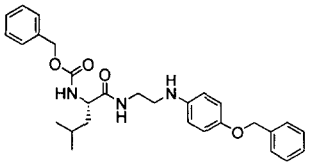
Glaxo-SmithKline



Axys-Merck



Novartis



- A primary lead compound as a potent cathepsin K inhibitor having an *Ki* at 26 nM
- Selectively targeted to cathepsin K by over 10-fold against cathepsin L, B, C, H, D and G
- At 1.4 uM, suppressed osteoclast-induced CTx release by 50% in pit assay
- In pharmacokinetic study, OST-1857 showed an good oral absorption.
- In thyroparathyroidectomized rats, OST-1857 significantly inhibited the increase of plasma calcium level induced by PTH.

Summary

- Cathepsin K is a attractive target for selectively and efficiently modulating the osteoclastic bone resorption.
- OST-1857 is a lead compound which is specifically targeted to cathepsin K and showed efficacy in TPTX rats.
- OST-compounds are in process of the preclinical study, joined by Yuhan research center.