osteoporosis. We have produced a small molecule inhibitor of human cathepsin K, OST-3964, that potently and selectively inhibits the enzyme with the subnanomolar range of IC50. We evaluated the bone resorption inhibitory potency of this agent in three in vitro systems including rabbit, rat and human osteoclast-mediated bone resorption assay .
OST-3964 demonstrated about 10 times more potent inhibition of neonatal rabbit osteoclast-mediated bone resorption than reference compound SB-357114. It also inhibited rat osteoclast-mediated bone resorption but in much higher concentration than in rabbit, reflecting the reported structural difference of cathepsin K between rat and human species. Neonatal rat osteoclasts are much smaller in size and the resorption activity is weaker than the neonatal rabbit one, and this is the first report to quantify the level of the biochemical marker of bone resorption in vitro in rat system, which is regarded as the more exact endpoint to assess the osteoclast resorption activity than commonly used resorption pit number or area. Finally, OST-3964 inhibited the human osteoclast-mediated bone resorption with similar potency to rabbit. Actually, human osteoclasts is difficult to obtain and we, through coculture of human peripheral blood monocytes and UMR-106 rat osteosarcoma cell line, made it possible.
These data show cathepsin K inhibition by OST-3964 results in a significant reduction of bone resorption in vitro and the further preclinical research is ongoing.

[PA1-8] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

A newly developed antiarrhythmic drug CW-2201 is ideal in treating atrial fibrillation

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An ideal antiarrhythmic agent would selectively prolong the action potential duration more in extraordinarily depolarized cardiac myocytes than in normal cells, and show tissue selectively. Previously, we found out that CW-2201, a benzopyran derivative selectively inhibited the hKv1.5 current expressing predominantly in human atrium without affecting the HERG current expressing mainly in ventricle. Additionally, CW-2201 inhibited the K+ current in isolated human atrial myocytes. From those results, we proposed that CW-2201 would be one of the leading compound in developing the ideal antiarrhythmic drugs for atrial fibrillation. In this study, we examined the effects of CW-2201 on the action potentials in rabbit heart using conventional microelectrode technique. CW-2201 prolonged the action potential durations of atrial, ventricular myocytes and Purkinje fibers in a dose-dependent manner. However, the effect of CW-2201 on atrial APD was frequency-dependent whereas the effect of CW-2201 on the APDs of ventricular myocytes and Purkinje fibers was not frequency-dependent. Additionally, CW-2201 induced hKv1.5 block was frequency-dependent and inhibits the human atrial K+ current. These results strongly suggest that CW-2201 could be an ideal compound for atrial fibrillation

[PA1-9] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Influence of CCCP on Catecholamine Secretion from the Perfused Rat Adrenal Medulla

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It has been shown that elevation of the intracellular free [Ca2+] ([Ca2+]i) triggers a wide variety of cellular functions and is a common mechanism by which external signals elicit cytosolic events (Clapharm, 1995, Berridge, 1997). An increase in [Ca2+]i can arise by release of Ca2+ from endogenous stores (mitochondria and endo/sarcoplamic reticulum) and/or influx of external Ca2+ across the plasma membrane. The membrane-permeable weak acids carbonylcyanide m-chlorophenylhydrazone (CCCP) and carbonylcyanide p-(trifluoromethoxy)phenylhydrazone (FCCP) are found to collapse the negative mitochondrial membrane potential that is the driving force for Ca2+ uptake (Gunter and Pfeiffer 1990). The contribution of mitochondria in shaping the histamine-induced Ca2+ increase was studied using ruthenium red and the two proton ionophores CCCP and FCCP in bovine adrenal chromaffin cells. Both mitochondrial uncouplers reversibly increased [Ca2+]i and induced an inward current leading to cell membrane depolarization (B?ing, 2001). Therefore, the present study was attempted to investigate the effect of CCCP

on secretion of catecholamines (CA) from the isolated perfused rat adrenal gland and to establish the mechanism of its adrenomedullary secretion.
The perfusion (0.31ml/min) into an adrenal vein of for 90 min resulted in great increases in CA secretions. Tachyphylaxis to releasing effect of CA evoked by CCCP was not observed by repeated perfusion of it. The net increase in adrenal CA secretion evoked by CCCP still remained unaffected in the presence of pirenzepine or chlorisondamine. However, the releasing effects of CA evoked by CCCP were depressed by *pretreament with pirenzepine, chlorisondamine, nicardipine*, TMB-8, and the perfusion of EGTA plus Ca2+-free medium. CA secretory responses induced by Ach, high K+, DMPP, and McN-A-343 were significantly enhanced in the presence of CCCP (3?0-5 M).
Taken together, these experimental results indicate that CCCP causes the rat adrenomedullary CA secretion in a calcium-dependent fashion, suggesting strongly that this facilitatory effects of CCCP may be mediated by both stimulation of the Ca2+ influx and Ca2+ release from cytoplasmic Ca2+ store.

[PA1-10] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

High Throughput Fluorometric Assay for Cathepsin S inhibitors

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Lysosomal cysteine proteases are involved not only in protein metabolism but also in tissue remodeling, hormone activation and antigen presentation. Among the known cysteine proteases, cathepsin S exists exclusively as a single-chain proteinase. It is also characterized uniquely by its high stability at neutral pH and bell-shaped pH-activity profile. Cathepsin S has received attentions due to its role in the pathogenesis of asthma, Alzheimer's disease, rheumatoid arthritis and other diseases involving tissue destruction. Recently, several evidences demonstrate that selective inhibition of cathepsin S could be a potential strategy for modulating the immune response in autoimmune diseases such as asthma and rheumatoid arthritis.
We established a fluorometric assay with recombinant human enzyme to explore cathepsin S inhibitors from in-house chemical libraries. The assay in the format of 96-well plate is easily adapted for high throughput screening. Therefore, our HTS system can be robustly applicable to the discovery of cathepsin S inhibitor owing to its high sensitivity, precision, accuracy, and stability.

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[PA1-11] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Preclinical studies of CKD-732, an antiangiogenic and antitumor agent.

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We have developed a novel water-soluble fumagillin derivative, CKD-732, and performed preclinical studies as an antiangiogenic antitumor agent. In endothelial cell proliferation assay, CKD-732 was found to show a 72 fold more potent activity compared to fumagillin. In addition, in the Matrigel assay, the hemoglobin content of Matrigel in CKD-732 treatment mice was less than 20% of that in control. Therefore, CKD-732 was found to effectively inhibit a neovessel formation through an angiogenic process. In tumor xenograft models, s.c. injection of CKD-732 induced the growth inhibition of PC-3, CX-1, SKOV-3, LX-1, SNU-16, MDA-MB-231 and Hep3B tumors in a dose dependent manner as much as 64, 74, 69, 69, 68, 70 and 65%, respectively. In animals bearing A375-SM and PC-3 tumors, CKD-732 induced stasis of tumor growth and displayed ILS of >200%. To evaluate the pharmacokinetic property of CKD-732, ADME studies were performed in vitro and in vivo. CKD-732 and 14 metabolites were found from the in vitro samples, and a major metabolite(M11) was identified as a N-oxide form. CKD-732 and M11 exhibited similar plasma kinetic profiles with linear pharmacokinetics, which were detected at 6~8 hrs after an i.v. administration in rat and dog. Therefore, CKD-732 was shown to be relatively stable and to have a long half-life in plasma. These results suggest that the strong antiangiogenic antitumor activity and the improved metabolic