High-throughput assays require that both the assay technology, as well as the instrumentation necessary to measure the results, be reliable and cost effective. cAMP and Tumor Necrosis Factor (TNF-alpha) are important mediators and indicators for a litany of cellular responses. As such, assays of these moieties are common in research and drug discovery high throughput screening (HTS). The ability to accurately measure changes in these compounds, using a homogeneous assay technology, saves considerable amounts of time and expense. Assays based on CoA's homogeneous time-resolved fluorescence (HTRF®) technology use a combination of time-resolved fluorescence (TRF) and fluorescence resonance energy transfer (FRET) to investigate biomolecular interactions. BioTek's Synergy® II Multi-Detection Microplate Reader combines a high intensity xenon flash lamp with deep blocking fluorescence filters to provide high sensitivity. The combination of a high performance multi-detection reader and a robust homogeneous assay technology provides for a reliable HTS solution. Here we describe the use of the Synergy 2, in conjunction with Gen5® Data Analysis Software, to quantify the signal and perform the data reduction for cAMP and TNF assays using HTRF technology from Cisbio. Examples of typical performance of these assays when measured on a Synergy 2 will be provided along with an overview of both the assay technology and reader design.

Toxicological evaluation of a new compound isolated from the root of Piper chaba

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Toxicology deals with the adverse effect of bioactive substances on living organisms. All drugs are toxic at higher doses. Therefore, in order to develop and establish the safety and efficacy level of a new drug, toxicity study is very essential experiment. No drug is used clinically without its clinical trial as well as toxicity study. Toxicological data helps to make decision whether a new drug is adopted for clinical use or not. In this work, a new bornyl ester of piperic acid (piperebol) isolated from Piper chaba was subjected to toxicological study. The compound piperebol was isolated from the root extract of Piper chaba by using various chromatographic techniques and its structure was determined using spectroscopic methods such as NMR, high resolution mass spectrometry. The compound piperebol (300 and 50 μg/day) was administered daily for 14 days in Swiss albino mice and the effects on body-weight, haematology and biochemical parameters of blood were studied. There was no significant change detected in haematology, blood biochemistry showed that SGPT, SGOT and SALP level decreased remarkably in comparison to control mice. Again histopathological study showed that this compound is toxic at higher doses (>300 μg/day) for liver, heart and kidney tissues. From the above study it can be said that the new compound piperebol may possess significant hepatoprotective effect. Therefore, further hepatoprotective activity study and dose adjustment may establish this compound as a safe hepatoprotective drug in modern medicine.

Toxicogenomic analysis of gene expression changes in MNNG-treated human lymphoblast TK6 cells

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Using toxicogenomics technique, we study the patterns of altered molecular expression that are caused by specific exposures or disease in a high throughput manner, which would reveal how the toxicant act and cause disease. Here we investigated the gene expression profile of the human lymphoblast TK6 cells treated with MNNG. We extracted total RNA at 2h from TK6 treated with MNNG (0, 25, 50, 100 μM) in three independent experiments and hybridized cRNA probes with oligo DNA chip (Applied Biosystems Human Genome Survey Microarray). We analyzed raw signal data with R program and AVADIS software and identified a number of regulated genes with more than 1.5 log-scale fold change and statistical significance. We extracted 14 genes including G protein alpha 12 showing deregulation by MNNG. And MNNG-induced toxicity might result in change of the signaling pathways for MAP kinase, Hedgehog, Wnt and insulin, regulation of actin cytoskeleton and tight junction and various metabolisms. Further studies will be needed to validate differential gene expressions and their biological meanings.

The effects of KR62980 on osteoblast differentiation and ovarioctomized mice by in vivo micro-computed tomography

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The PPAR-γ is an adipocyte-specific transcription factor and negative regulator on osteoblast differentiation of bone marrow stromal cells. We investigated whether KR62980, a novel PPAR-γ agonist exhibits any effects on osteoblast differentiation and bone formation. KR62980 induced alkaline phosphatase (ALP) activity and increased extracellular matrix calcification in mouse calvaria-derived osteoblasts. RT-PCR analysis revealed that KR62980 also increased the mRNA transcripts of specific genes involved in osteoblastic differentiation, including BSP, ALP, Runx-2, and ostein in cells. However, KR62980 did not upregulate PPAR-α and ap2 associated with adipocyte differentiation. Our results indicate that KR62980 exerts its effects of bone metabolism to spare bone mass not only as an activator of osteoblastogenesis but also as an inhibitor of adipo genesis. In addition, using an ex vivo neonatal calvaria implantation system, we showed the osteogenic activity of KR62980. Histological examination confirmed new bone formation in mice transplanted with paper treated with KR62980. Bone mineral content and density were measured in femur by Micro CT. KR62980 treatment increased BMC and BMD of femur to a greater extent in ovarioctomy of mice. Bone marrow cells from each group were used to isolate osteoblasts and cultured with osteogenic factors for 20 and 40 days. KR62980 treated group exhibited the highest level of calcium mineralization, as assessed by Alizarin Red staining, and ALP activity using ALP activity staining kit. Taken together, these results suggest that KR62980 may have potential as a drug candidate for treating bone diseases in vivo and in vitro.

The design of an enantiomer analogue for proteolytic resistance of pleuromicrocidin derived from Pleurocetes americus

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Pleuromicrocidin(Ple) is a 25-residue peptide which is derived from the skin mucus secretion of the winter flounder (Pleurocetes americus). In the present study, we investigated antibacterial activity and its mode of action of Ple on human pathogens fungi. Ple showed potent antifungal activity with low hemolytic activity. To investigate the antifungal mechanisms of Ple, the cellular localization and membrane interaction of Ple were examined. Confocal fluorescence microscopy showed that FITC-labeled Ple accumulates in the plasma membrane of C. albicans. Moreover, proteolysis regeneration and membrane-disrupting activity by DPH-labeled membrane support the idea, that Ple exerts fungicidal activity against C. albicans with the disruption of a plasma membrane. To aim for which was application of a therapeutic agent, we designed a synthetic enantiomeric peptide composed of all-D-amino acids to enhance proteolytic resistance. For proteolytic studies, HPLC analyses were performed. To examine the susceptibility of all-D-Ple towards proteolysis, some proteases were studied in parallel to that of all-L-Ple. The all-D-enantiomer remained intact after the incubation with the enzymes, while the all-L-enantiomer was sensitive. Additionally, its antibacterial activity showed proteolytic resistance. Therefore, these result indicated that a therapeutic potential of all-D-Ple with regard to its proteolytic resistance against human fungal and bacterial infections.

The antimicrobial effect of C-terminal amidation of antibiotic peptide, PMAP-23

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The antimicrobial peptide, PMAP-23, a member of the cationic family, has potent killing activities against a broad spectrum of microbial organisms, did not characterized clearly. Here, we try to characterize the C-terminal non-amidation and amidation of PMAP-23 with membrane phospholipids. The observed structural changes in membrane environment and antimicrobial activities against microbial cells were similar. But in gram-negative bacteria, membrane depolarization and membrane permeation assays, as well as electron microscopy, suggest that two peptides have different characters within membrane permeability. While the amidation peptide can penetrate in outer membrane and inner membrane of E. coli, the non-amidation peptide did not penetrate in inner membrane. The latter may indicate to be resulted by LPS, major component of outer membrane in gram-negative bacteria.