P2E-V-022  A novel time-resolved fluorometric immunosay for screening of antichlamydial compounds
Laura Riihimaki1, Pirvi Tampulma1, Jioni Alvesalo1, Sari Airinen1, Maija Leinonen1, Perti Hurskainen1, Kristian Enkvist1, Pia Vuorela1
1Vitki Drug Discovery Technology Center; Fac. of Pharmacy, Univ. of Helsin-ki, Finland; 2National Public Health Inst., Dept. in Oulu, Finland; 3PerkInElmer Life and Analytical Science/Waltham, MA, USA, Finland.

Purpose: Chlamydia pneumoniae is a human pathogen causing acute respiratory infections as well as chronic infections, which have been implicated e.g. in the pathogenesis of atherosclerosis and asthma. We developed a novel technological approach to detect intracellular bacteria using time-resolved fluorometric immunosay (TR-FIA) and optimized this method for susceptibility testing of chlamydia.

Methods: In this cell-based, 96-well plate assay chlamydial inclusions are labeled with europium-conjugated antibody, and quantified as time-resolved fluorometric signals by means of a multilabel counter. To confirm the reliability of the TR-FIA, susceptibilities of C. pneumoniae reference strain to a set of antimicrobial agents were determined by the TR-FIA, conventional immunofluorescence staining and real-time PCR.

Results: MICs measured using the different methods demonstrated a good correlation. Rifampicin and erythromycin were the most effective antimicrobial agents, with MICs of 0.004-0.008 and 0.016 μg/ml. Doxycycline and minocycline MICs varied between 0.031 and 0.063 μg/ml. With ciprofloxacin the MICs obtained using the TR-FIA and PCR (0.333 μg/ml) and IF staining (1.0 μg/ml). For ofloxacin MICs lay between 0.5 and 1.0 μg/ml. Penicillin G and streptomycin did not inhibit chlamydial growth. Data relating to reproducibility (day-to-day variation 9.0%) and the signal-to-background, signal-to-noise and Z′ values (6.5, 6.9, and 0.4, respectively) showed the suitability of the TR-FIA for screening.

Conclusions: The TR-FIA is considerably less labor-intensive, reliable, and objective, new alternative for detecting chlamydia in vitro which offers significantly higher throughput compared to previous methods and is thus especially suitable for screening of new antichlamydial compounds.

P2E-V-024  Attenuation of post-ischemia reperfusion injury by LS-NTU-106 (an aporphine alkaloid) in rat hearts
Wei-Luen Chang, Ming-Jai Su
Inst. of Pharmacology, Coll. of Medicine, National Taiwan Univ., Taiwan.

Purpose: Previous studies in our lab have indicated that pretreatment of LS-NTU-106 before ischemia could reduce the ischemia and ischemia/reperfusion injury. However, Reperfusion therapy has become a practical and effective strategy in the salvage of ischemic myocardium. The objective of this study was further to evaluate whether LS-NTU-106 administered at the time of coronary reperfusion could have the same cardioprotection as pretreatment before the ischemia reperfusion period.

Methods: Anesthetized, open-chest rats were subjected to 60 min of regional ischemia and 120 min of reperfusion. Regional ischemia could induce severe arrhythmia including ventricular tachycardia and ventricular fibrillation. Animal with similar arrhythmia pattern were randomly received vehicle or LS-NTU-106 10 mg/min before the onset of reperfusion only.

Results: Infarct size was reduced in the LS-NTU-106 treated groups in a dose-dependent manner compared with control. (LS-NTU-106 10⁰ mole/kg 22.60±2.23%; vs LS-NTU-106 10⁷ mole/kg 3.15±3.26%; vs control 43.57±2.74% P<0.05). It also reduced plasma concentrations of creatine kinase and cardiac MPO activity. After 120 min reperfusion recovery of developed pressure was 78.2±16 mmHg and 90.5±11 mmHg in control and LS-NTU-106 (10⁷ mole/kg, 1) treatment group. Recovery rates of pressure development (+dp/dt) and relaxation (-dp/dt) also significantly improved in hearts treated with LS-NTU-106.

Conclusions: These protective effects afforded by LS-NTU-106 from post-ischemia reperfusion injury were abrogated by opioid receptor antagonist, naloxone and 1,KATP blocker 5HD.

P2E-V-025  Inhibition of preadipocyte differentiation by Oren-gedoku-to treatment in 3T3-L1 cultures
Kunihiro Suzuki, Motoko Chino, Kiyoshi Sugiyama
Fac. of Pharmaceutical Sciences, Hoshi Univ., Japan

Purpose: Obesity is characterized by an increase in the number and size of adipocytes. The number of the adipocyte is determined by the number of preadipocytes, and also determined by the differentiation rate from preadipocyte into adipocyte. The differentiation of 3T3-L1 preadipocytes to adipocytes is useful as a model of adipocyte differentiation. Kampo formulation, Oren-gedoku-to is a traditional herbal medication used in Japan for many centuries to treat hyperlipidaemia, hyperlipidaemia, and diabetes. We examined the effect of Oren-gedoku-to and its components on adipocyte differentiation of 3T3-L1 cells.

Methods: 3T3-L1 cells were cultured in DMEM containing 10% FBS. The differentiation to mature adipocytes was induced by the addition of 0.5 mM 3-isobutyryl-1-methyloxanthine (IBMX), 1.0 μM dexamethasone, and 5.0 μM insulin to confluent 3T3-L1 preadipocytes for 2 days. At 5 days after confluence, the accumulation of lipid in the cytoplasm of differentiated adipocytes was observed by oil red O staining. Adipose conversion was quantified by the measurement of glyceraldehyde-3-phosphate dehydrogenase (GPDH), a marker enzyme of adipocytes. Oren-gedoku-to and its components were added into cells for 6 days during differentiation after confluence.

Results: Oren-gedoku-to treatment (0.1-0.2 mg/ml) significantly decreased GPDH activity and oil red O staining compared to controls that did not receive Oren-gedoku-to. Oren-gedoku-to is composed 4 herbal medicines. We investigated the effect of the herbal medicine, Oren-gedoku-to, on adipocyte differentiation of 3T3-L1 cells. Of 4 herbal medicines tested, Oren (Coptidis Rhizoma) and Obaku (Phellodendri Cortex) inhibited the differentiation of 3T3-L1 preadipocytes to adipocytes. The effect of chemical constituents of Oren and Obaku on the differentiation of 3T3-L1 cells was examined. The major chemical constituent of Oren and Obaku, berberine strongly inhibited the adipose conversion of 3T3-L1 cells at 3.71-0.371 μg/ml. From the content of berberine in Oren-gedoku-to, it is very likely that berberine plays an important role in the differentiation inhibitory effect of Oren-gedoku-to.

Conclusions: These data indicate that Oren-gedoku-to, Oren, Obaku, and berberine inhibit the increase in the number of adipocytes. Thus, Oren-gedoku-to might be effective therapy for hyperlipidaemia, hyperlipidaemia, and diabetes.