Screening and Confirmation of Designer Drugs and Anorectics in Urines using Immunoassay and GC/MS

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Immunoassays are frequently used for an screening method to detect the presence of drugs in Urine. The main advantages of the method are well known -- simplicity of handling samples, rapidity, sensitivity, and specificity of analysis. However, it is also known that immunoassays exhibit cross-reactivity to related drugs and there are only limited specific immunoassays on the market. This study reports on the ability of TDx to detect urine samples obtained from suspects of taking over-the-counter medications and illegal drugs containing ATS, designer drugs. Samples identified as positive or negative by TDx assay were confirmed by GC/MS. Accusign MET, SD bioline, TDx, Solaris and selectra were also compared respectively in terms of the specificity and sensitivity for drugs. First, MDMA and MDA were detected in 4 samples, and only MDA was detected in 1 sample. Second, ephedrine (EP) and pseudoephedrine(PEP) were detected in 9 samples, and methoxyphenamine(MTP) was detected in 1 sample. Third, 6 phentermine(PT), one fenfluramine(FF) and two Phendimetrazine(PDT) were detected from the 24 samples. This study also describes the following results for 15 drugs with 6 kinds of immunoassays. First, 250ng/mL of MDA, MDMA, MDEA, EP, norEP, and norPEP were positive by Solaris. Second, the sensitivity for MDMA was the highest by TDx The sensitivity order was MDMA>MDEA>FF>PT>MPT. Third, FF was the most sensitive by Selectra. Fourth, the sensitivity for MDA was high by SD-line AMP, while the sensitivity for MDMA was high by SD-line MET. Fifth, the sensitivity for MDMA and MDEA was high by Accusign, but the sensitivity for MDA was very low.

Poster Presentations - Field B1. Physiology

[PB1-1] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Effects of Protein Kinase Inhibitors on Histamine Release and ROS Generation in RBL 2H3 Cells

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Previous report showed that histamine release by HCl was mediated via reactive oxygen species (ROS) generation in RBL 2H3 cells. To investigate action of protein kinase on histamine release and ROS generation, we observed effects of protein kinase inhibitors on histamine release and ROS generation in RBL 2H3 cells stimulated by HCl. HCl dose-dependently increased both histamine release and ROS generation. HCl-induced histamine release was significantly inhibited by bisindolmaleimide (10 μM), DHC (10 μM), and wortmannin (10 μM), but not by PD098059 (10 μM). On the other hand, HCl-induced ROS generation was significantly inhibited by DHC (10 μM), but not by bisindolmaleimide (10 μM), wortmannin (10 μM) and PD098059 (10 μM). However KN-62 did not inhibited both. These results showed that involvement of protein kinase in regulation of histamine release and ROS generation may be different and only tyrosine kinase may be associated with regulation of both histamine release and ROS generation in RBL 2H3 cells.

[PB1-2] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Protective effect of KR-32000 against hypoxia- and oxidative stress-induced cardiac cell death

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A benzopyranyl derivative, KR32000, synthesized as a plausible KATP opener, has been shown to exert cardioprotective effect in vivo myocardial infarct model. In this study, we investigated whether KR32000 can produce cardioprotective effect against hypoxia- and reactive oxygen species(ROS)-induced injury in heart-derived H9c2 cells. Hypoxic injury was induced by incubating cells in anaerobic chamber (glucose-free, serum-free DMEM, 85% N2, 5% CO2, 10% H2) and oxidative stress was induced by buthionine sulfoximine(BSO). Cell viability was evaluated by MTT and LDH assay. KR32000 30 μM significantly decreased LDH release induced by hypoxia in H9c2 cells. This decrease in LDH release was inhibited by HMR1883, a blocker of sarcolemmal KATP channel, but not by 5HD, a blocker of mitochondrial KATP channel. KR32000 also decreased BSO-induced H9c2 cell death. ROS generation by BSO was decreased by 30 μM KR32000. These results suggest that KR32000 protects H9c2 cells from hypoxia- and oxidative stress-induced injury, at least in part, through sarcolemmal KATP channel and antioxidant effect. And also, we measured BSO-induced ROS generation to confirm whether KR32000 had protective effect from ROS-induced cardiac injury.
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[PB1-3] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Effect of Chitosan Oligosaccharide on Tyrosinase Activity

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Tyrosinase (monophenol, 3,4-β-dihydroxyphenylalanin oxygen oxidoreductase, EC 1.14.18.1), which plays a pivotal role in melanogenesis. It is single chain glycoprotein catalyzing the hydroxylation of tyrosine to β-3,4-dihydroxyphenylalanin (DOPA) and the oxidation of DOPA to DOPA quinone. To investigate whitening effect of chitosan oligosaccharide, we obtained chitosan oligosaccharide [(glucosamine)2-6] by NaNO2 oxidation and measured the effect of chitosan oligosaccharide on tyrosinase activity. Chitosan oligosaccharide dose-dependently inhibited tyrosinase (2 unit) activity and inhibited by 18.8% at dose of 100 μg/ml. Vitamin C, arbutin and kojic acid that are well known to be inhibitor of melanin production dose-dependently inhibited tyrosinase (2 unit) activity. These results suggest that chitosan oligosaccharide may be used as inhibitor of melanin production in melanocyte, which will be further studied.

[PB1-4] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Quercetin analogs extracted from Lidera erythrocarpa protects heart-derived H9c2 cells from oxidative stress-induced death

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Bioflavonoids are semi-essential food components that are ubiquitously present in nature. It has been reported that flavonoids act as anti-oxidant as well as anti-cancer agents. Quercetin is one of the most widely distributed bioflavonoids in the plant kingdom. The goal of this study was to investigate effects of quercetin analogs extracted from Lindera erythrocarpa, quercetin 3-O-α-arabinofuranoside and quercetin 3-O-α-L-rhamnoside, on oxidative stress-induced cell death. Cell death was induced by using BSO, buthionine sulfoximine, which inhibit GSH level and subsequently increase ROS level. Cell death was quantitatively determined by measuring lactate dehydrogenase(LDH) activity, by propidium iodide(PI)-uptake. The intracellular level of ROS was measured by using DCFH-DA.
BSO-induced LDH release and PI-uptake was significantly decreased by quercetin 3-O-α-arabinofuranoside and quercetin 3-O-α-L-rhamnoside. These components also reduced ROS production induced by treatment with BSO. In conclusion, our results suggest that quercetin 3-O-α-arabinofuranoside and quercetin 3-O-α-L-rhamnoside can protect heart-derived H9c2 cells from oxidative stress-induced death through antioxidant effect.
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