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Development of In Vitro Cellular Circadian Rhythm Model to Test the Effects of Neurotoxicants on Circadian Rhythm

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Introduction: The goal of this study is to develop an in vitro cellular reporter assays to determine cellular circadian rhythm in neural cells using circadian reporter vector. We will then use this in vitro system to 1) test known neurotoxicants for their effects on circadian rhythm, 2) determine if antioxidants that were known to reduce neurotoxicity can prevent the loss of, or restore a normal pattern of cellular circadian rhythm.

Materials and Methods: Stably transfected U87 MG (human glioastroma cell line) circadian reporter cells were prepared by transfection with hPER2 promoter-driven dLuc expression vector using transfection reagent followed by selection of stably transfected cells with antibiotic G418. In vitro cellular bioluminescence assay was performed in the reporter cells to determine cellular circadian rhythm. After starvation for 3 days, cells were synchronized with 50% horse serum and then maintained in recording medium w/wo treatment. The luminescence signal was determined from the live cells using LumiCycle 32 and analyzed with LumiCycle Analysis software. Cells were treated with chemicals which are known as neural toxicants [pesticides:MPP+iodide (MPP+); Chlorpyrifos (CPF); 4,4'- DDT (DDT), veterinary drugs: cefazolin (CFZ); ciprofloxacin (CPX); polymyxin B (PMX), heavy metal: methylmercury (Hg)], with or without antioxidant, methylselenocysteine (MSC) or n-Acetylcysteine (NAC), for 5 days in recording medium.

Results: Using this in vitro model, we found that a battery of known neurotoxic agents (pesticides: MPP+; CPF; and DDT, heavy metal: methyl mercury, veterinary drugs: CFZ; CPX; and PMX) disrupted cellular circadian rhythm with dose dependence. More interestingly, antioxidants such as MSC and NAC that were known to reduce neurotoxicity can prevent the loss of, or restore a normal pattern of cellular circadian rhythm.

Conclusions: Given the lack of human data on how disruption of circadian rhythm contributes to neurotoxicity, there is a critical need to establish and validate an in vitro circadian reporter assays in neural cells. This in vitro model would be a valuable tool to screen and classify neurotoxicants in terms of circadian disruption. It also can be used to investigate the impact of circadian disruption on neural cytotoxicity.

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Anti-prion activity of *(E)-3,4-dihydroxystyryl 2,3,4-trihydroxybutanoate* against BSE-associated prion protein

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Introduction: Bovine spongiform encephalopathy (BSE) is an infectious neurodegenerative disorder in cattle, which is characterized by the accumulation of an abnormal form of prion protein (PrP^{BSE}) that is partially resistant to protease digestion. Although several researchers have been searching for anti-prion drugs to block the conversion process, there are currently no proven therapeutic agents for BSE. Thus, it is important to identify inhibitors with therapeutic or prophylactic activity against BSE prion. In a previous experiment, we found the new anti prion natural products (*Rubus coreanus Miquel*, *Zinnia elegans and Lagerstroemia indica*) that can inhibit the formation of PrP^{BSE} in a persistently BSE infected cell line M2B. In this study, we investigated the prion inhibition ability of *(E)-3,4- dihydroxystyryl 2,3,4-trihydroxybutanoate* isolated from *Lagerstroemia indica* ethanol extracts in M2B cells. Based on the analysis of PrP^C-GN8 (a known anti-prion compound) complex structure, we suggested essential PrP residues responsible for the interaction of PrP^C and *(E)-3,4- dihydroxystyryl2,3,4-trihydroxybutanoate.*

Materials and Methods: Approximately 5,000 M2B cells were seeded to each well of 96-well plate prior to the addition of compound. And the following day, we added 50㎕ of the diluted products in the range from 200 *uM* to 1.56 *uM* to each well on every passage. The cells were incubated for 3 days and passaged six times. In order to confirm inhibitory concentration, the Standard Scrapie Cell Assay (SSCA; Klohn *et al.*, 2003) was applied. The SSCA result was further confirmed using IDEXX HerdChek® ELISA and western blot analysis (WB) as previously described (*Tark et al.*, 2015). Cell viability was evaluated using the CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (MTS assay, Promega, USA). We also investigated the binding mode and interaction between the compound and prion protein in the normal conformation (PrP^C) for the prion hotspot.

Results: We measured the inhibitory effect of anti-prion compound using three methods [SSCA, WB and ELISA]. The minimum inhibitory concentration was 45 *uM* of

(E)-3,4-dihydroxystyryl2,3,4-trihydroxybutanoate in SSCA. We also observed that this compound showed inhibitory effect against PrP^{BSE} by WB and ELISA. In *in vitro* model, the compound was confirmed to having the non-cytotoxicity by MTS. Molecular docking results provided structural models and binding affinities for the interaction between PrP^{C} and *(E)-3,4-dihydroxystyryl 2,3,4-trihydroxybutanoate*.

Conclusions: We have identified one compound from *Lagerstroemia indica* as an effective inhibitor for formation of PrP^{BSE} in BSE infected cell culture model. Further experiments in animal models will be necessary to confirm the effect of this compound *in vivo*.

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Comparisonof Newcastle disease vaccines administered as different spraying machines inrelation to immune response and protective efficacy in day old chicks

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Introduction: Newcastledisease (ND) is one of the most devastating poultry infection resulting economicloss to the poultry industry. Spray vaccination to young birds is a commonpractice and even duty in many countries. However, there is little known about theeffect of spray particle size which could affect the efficacy and safety of thevaccine. In this study, we used four ND vaccine strains to compare the immuneresponse and protective efficacy when three different spraying machines appliedto a day old chicks.

Materials and Methods: Ten 1-day-oldSPF chicks per group were immunized with $10^{5.0}EID_{50}$/chickof the NDV B1, K148/08, VG/GA, and DSB-HP strains using 3 types of sprayerswith different particle sizes. At 3 days after immunization, 3 chicks in eachgroup were subjected to evaluate histological lesions in lung. Two weeks afterimmunization, all chicks were challenged with velogenic NDV. Clinical signs anddeath were scored to evaluate the protective efficacy of the vaccines. Mean viralshedding levels and HI titers of the sera were also determined on the plannedschedule.

Results: In thehistological examination, when the NDV B1 strain were immunized by a finesprayer, an extent of inflammation reaction were observed. Generally, theclinical scores from chicks immunized with course sprayer were higher thanthose from the chicks immunized with fine sized sprayer. Interestingly, Chicksimmunized with the NDV K148/08 showed significantly higher viral shedding fromthe cloaca compared to other strains. There was a tendency that the vaccinewith finer spray particles induced higher HI titer and showed higher survivalrates after challenge.

Conclusions: In this study,we did not measure the exact particle size of one sprayer which has novalidated data. However, it supposed that when higher pathogenic vaccine strainis administered with lower particle sprayer, excessive immune reaction can beinduced due to its deposition in the lower respiratory tract. Therefore, itseems important to choose an appropriate spraying machine and the vaccinestrain to maximize the vaccine efficacy.

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An International Collaboration to Trace Movement Route of Migratory Birds

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Introduction: GPS tracking device on wild birds allows scientists to have an accurate and complete features of their migratory pattern. A collaboration of Animal and Plant Quarantine Agency (QIA) of Korea, Chinese Academy of Science (CAS), and Korea Institute of Environment Ecology (KIENV) is ongoing.

Materials and Methods: A total of 14 devices with GPS tracking system, provided by the QIA, were attached to captured wild birds at Poyang lake of Jiangxi Province of China on December 2015 (11 heads) and March 2016 (3 heads). The on-site attachment operation was performed by CAS with technical assistance of KIENV. The record of movement was monitored by 'Migratory Bird Tracking System (www.tspmap.kr) of the QIA.

Results: As of September 2016, movement way of eleven birds was being traced in real time. Some of them showed their migration toward northeast up to Russia through Heilongjiang Province. The others flied to northwest and stayed in Mongoila.

Conclusions: This study of tracking route proved areas where birds to wintering (i.e. Southern China, Korea) and those to reproducing (i.e. Northen China, Russia, Mongolia) sites are crossed. This area can be a potential epicenter of spread transboundary animal disease such as highly pathogenic avian influenza.