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鋰鹽參與免疫調解之機轉 (1/2)

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行政院國科會專題研究計畫成果報告(期中報告)

鋰鹽參與免疫調節之機轉(1/2)

Mechanisms of Lithium in Immunomodulation (1/2)

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(1) For the past year, the H_2O_2 induced apoptosis of Jacket and HL-60 cell line were investigated. HL-60 (1 x 10^6 cells) were cultured in the 24-well plate for overnight. Quercetin (20, 30, 40, 50 μM; a gift from Dr. CY Chen from Taipei Medical University) were added to the culture plate for 12 hour. Quercetin is well known to induce apoptosis in HL-60 cell, therefore, we used as a positive control for the apoptosis assay. HL-60 treated cells were harvest, and DNA ladder were performed. Briefly, DNA lysis buffer were added to the tube and incubate the tubes for 56^{o}C overnight, RNAase were added and phenol/chloroform were used for extraction DNA. DNA ladder were visualized by 2% agarose gel electrophoresis. However, the effect of H_2O_2 induced apoptosis in Jacket cell and HL-60 were not obvious, and the proposed anti-apoptosis effect of Li on these cell could not clearly seen. The various conditions on these cells are still under investigation.

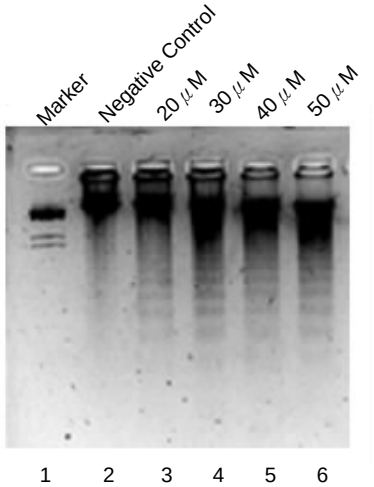

Fig. 1. Quercetin induced apoptosis of HL-60 cell.

Quercetin (20, 30, 40, 50 μM corresponding to lane 3, 4, 5, 6) were added to the HL-60 cell (1 x 10 6 cell) for 12-hour. DNA were extracted and

DNA ladder were observed.

(2) Primary rat microglia cell culture

Therefore we have set up the microglia primary culture from rat brain with the assistant of Dr. Ching-His Wu at National Defense Medical Center. The primary mixed glia cultures were prepared from the brains of 7-day-old Wistar rat. Briefly, cells from the whole brain were dissociated in RPMI-1640 medium, supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin/streptomycin. After 10-14 days culture, the flasks were shaked and the supernatant contained glia cells were collected. Cells were seeded at 1.4 x 10^4 cells/well/1ml on 24-well plate and incubated for overnight until the cells attached to the plate. Different concentrations of the Li (0.5 1, 3, 5, 10 mM) were added to the plates for 3 or 7 days. Then LPS were (1 μg/ml) added to the plate for microglia activation, 1 day after, the supernatant were collected and the viability of the cell were studied.

The primary microglia culture are in stable condition now and microglia cell markers were tested. Final culture of the primary microglia were over 95% purity by staining with microglia-specific epitope OX-42.

(3). Measurement of cytotoxicity

The mitochondrial dehydrogenease activity which reduces 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma) in active mitochondria to purple formazan were used to determined cell survival in a colorimetric assay. Briefly, the 24-well plate were culture with 1 x 104 cells until confluent. For lithium treatment, different concentration of LiCl (0.5, 1, 3, 5, 10 mM) will be added before addition of LPS (1 μg/ml). After 24 hours treatment medium will be removed and 30 μl of MTT will be added to the wells for 4 hour with shaking. DMSO will be added to the well and for each point, the 100 μl supernatant will be added to the 96-well plate with triplicate and measured at 570 nm wavelength by ELISA plate reader. The OD will calculate reference to control.

The MTT assay for microglia cells after Li addition were measured, all of them were over 90% survival. Therefore the addition of Li and LPS will not affect the survival of the microglia cell culture

(4) Cytokines (IL-6, TNF-α, TGF-β, IL-10) analysis by ELISA

Microglia cells ($1x10^4$ cell) were cultured in the 5% CO2 incubator for 24

hours and different concentrations of LiCl (0.5, 1, 3, 5, 10 mM) were added (3 or 7 days for long-term treatment) before addition of LPS (1 μg/ml). After incubation, the cells were harvest by centrifugation and the supernatant were collected for the assay. ELISA were performed according to the manufacture procedure (R & D System Inc.). Briefly, the monoclonal antibody to each cytokines were coated to the 96-well plates at 4^{o}C overnight. Then the plates were blocked by 1% BSA in PBS at room temperature for 1-2 hour. After blocking the plates were washed for 3 times, and the patients culture supernatant with proper dilution were added to the plates and incubated at 37^{o}C for 2 hours. The secondary antibody conjugated with enzyme were added to the plates and incubated for another 2 hours. After 3 times washing, the substrate (TMB) were added and the intensity of the plate will be stopped (1N HCl) as compared to the standard. The absorbances of plates were measured by ELISA plate reader at 450 nm. The concentration of the cytokines were calculated according to the standard curve.

The Li alone (0, 0.5, 1, 3, 5, 10 mM) will not affect the IL-6 production, but 5 mM and 10 mM of Li will attenuate (521.1 and 557.6 pg/ml, respectively) LPS-induced IL-6 production (2068 pg/ml) from primary rat microglia cell. The effect of Li on LPS-induced cytokines production and signaling pathway are still under investigation. Carbamazepine, clopapine, and valproic acid will be test side by side for comparison.