

Effects of *Ganoderma lucidum* on Human Leukoeytes

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Abstract

Methanolic extracts of *Ganoderma lucidum* were treated on human peripheral blood mononuclear cell (PBMC) culture system in the presence of various immunostimulating or immunosuppressive agents. Phytotemagglutinin (PHA) induced cell proliferation was significantly inhibited by the GLE fraction that is the neutral components of the methanolic extracts of the carpophores. 12-O-tetradecan-oylphorbol 13-acetate (TPA)-Induced cell proliferation was inhibited by the fractions of GLA, GLC, GLE and GLG. However none of these fractions inhibited proliferation of the PBMCs stimulated with TPA plus ionomycin (IM). Treatment of the PBMCs with cyclosporin A (CsA) greatly blocked cell proliferation. When the cells were cultured with the methanolic fractions in the presence of CsA, concentration dependent inhibition of the cell proliferation was observed by the addition of GLC, GLE and GLG fractions. On the contrary, GLH fraction recovered the CsA induced inhibition of the cell proliferation. Taken together, among the methanolic fractions, GLE showed the highest inhibitory activity. This fraction might inhibit protein kinase C signal pathway and accelerate CsA signal pathway.

Summary of Results

We examined the effects of the methanolic extract of *G. lucidum* as well as its fractions partially purified by the polarities in combination with pH change on the proliferation of cultured human peripheral blood mononuclear cells in the presence of immunostimulating or immunosuppressing reagents.

Fractionation of *G. lucidum*

The methanolic extract, GLA, of the carpophores fractionated into GLB (lipophilic fraction), (hydrophilic), GLE (neutral), GLF (acidic), GLG (basic) and GLH (amphoteric).

Effects of PHA stimulation

Density gradient isolated human PBMCs (2×10^6 cells/well) were cultured with either 10 or 100 m g/ml of the methanol extracted fractions of *G. lucidum* in 96-well microculture plate at 37° C, 5% CO₂, in the presence or absence of PHA. The total methanolic extract, GLA, showed a slight inhibition of cell proliferation at 100 m g/ml. All the other fractions, as being partially purified by the process of fractionation, led to stronger inhibition of cell proliferation. At the concentration of 100m g/ml, four fractions such as GLC, GLE, GLG and GLH, led to significant inhibition of PHA-induced cell proliferation to 76% ($p < 0.05$), 14% ($p < 0.01$), 48% ($p < 0.01$) and 64% ($p < 0.01$) of that of the control, respectively. Therefore, GLE blocked Tlymphocyte mediated cell proliferation most efficiently at 100m g/ml. However at a lower concentration, 10m g/ml, GLE showed undetectable inhibition proliferation. In contrast to this, both GLG ($p < 0.01$) and GLH ($P < 0.05$) fractions significantly inhibited cell proliferation at this lower concentration.

Effects of TPA stimulation

Cells were cultured with fractions in the presence or absence of 20m g/ml of TPA. At 10m g/ml concentration, most of the fractions were ineffective in stimulating or inhibiting the cell proliferation except GLG whose inhibition was 82% ($p < 0.05$) of that of TPA alone. However, at 100m g/ml, four fractions such as GLA, GLC, GLE and GLG suppressed cell proliferations to 75% ($p < 0.01$), 77% ($p < 0.01$), 56% ($p < 0.01$) and 53% ($p < 0.01$) of that of TPA alone, respectively. Therefore protein kinase C signal transduction in human PBMCs was blocked by the neutral and basic fractions of the methanolic extract of *G. lucidum*.

Effects of co-stimulation with TPA and IM

Co-stimulation of the PBMCs with TPA and IM induced 9.6-fold increase of cell proliferation when compared with that of the medium alone. None of the fractions affected cell proliferation in the presence of TPA plus IM.

Effects of CsA stimulation

Treatment of the cells with 10 nM CsA led to great inhibition of cell proliferation. Five fractions of GLA, GLB, GLC, GLE, GLF, and GLG further inhibited cell proliferation significantly at 10m g/ml or 100m g/ml. GLD inhibited cell proliferation only at 100m g/ml. Interestingly only GLH recovered the inhibition of the cell proliferation in a concentration dependent manner. According to the FACS analysis of human PBMC treated with GLE for 5 days, 91% of the cells remained in the G₀/G₁ phase, 2% in the S phase, and 7% in the G₂/M phase of the cell cycle, suggesting that GLE blocks somewhere prior to the S phase of the cell cycle.

Based on our results, most of the fractions except GLE had mild activities as described in Shen-Nong-Ben-Cao-Jing. This property of mild activity confer this mushroom to the highest class. It could not be classified as tonic medicine of this mushroom if it had strong activities. Our results present the evidence of this mushroom being useful for immunomodulating traditional medicine. Furthermore, this study has great meaning in that normal human leukocytes were used in this experiment.



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