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Role of Protein Kinase-C and Rho Kinase in the Cytotoxic Effects of Bitter Melon Extract on Metastatic Breast Cancer Cells

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Introduction

Bitter melon is known to enhance uptake of glucose and lipid by healthy cells, and it is also known to inhibit growth of cancer cells [1,2,3]. However, the effects of bitter melon extract (BME) for inhibiting uncontrolled division of cancer cells depend on the types of cancer cells. Since breast cancer is one of the most common cancers in women [4], breast cancer cell line (MCF-7 cells) has been chosen in this project to investigate the effects of BME.

Rho Kinase (ROK) and Protein Kinase C (PKC) are critically involved in cell division, cell migration, and cell survival [5,6]. Figure 1. ROK phosphorylates MYPT1 [Myosin targeting subunit of myosin light chain phosphatase (MLCP)] and inhibits MLCP. PKC phosphorylates CPI-17 which then binds with MLCP. Thus, ROK and PKC both inhibit MLCP activity favoring balance shift towards enhanced myosin light chain (MLC) activity which then phosphorylates myosin light chain (MLC). Phosphorilated MLC activates actin-myosin cross bridge formation which in turn regulates cellular processes such as cell migration, cell division, and cell survival. However, roles of ROK and PKC inhibition on MCF-7 cells have not been established. In addition, whether the effects of BME are mediated by ROK or PKC are unknown. Thus, we aimed to investigate if BME exerts cytotoxic effects on breast cancer cells (MCF-7 cells) and if PKC and ROK mediate BME’s effects.

Experimental Procedures

1. Making bitter melon extract (BME) Fresh bitter melons were purchased from an Asian grocery store. The melons were washed and ground in a juice extractor. The juice was centrifuged at 5000 RPM (15 min) and filter sterilized. BME aliquots were stored at -80°C.

2. Dose-response effects of BME Equal number of MCF-7 cells were plated in 250 mL culture flasks containing DMEM medium and increasing amount of BME [0%, 0.5%, 1%, 2%, 5%, and 10% of BME (v/v)].

3. Testing roles of Rho kinase (ROK) and protein kinase C (PKC) inhibition To study whether PKC or ROK play any in mediating BME’s effects, equal number of MCF 7-cells were cultured in 100 mL culture flasks containing DMEM medium in the following conditions: 0% BME, 1% BME, PKC inhibitor (GF109203x; 0.5µM); ROK inhibitor (H-1152; 1 µM), and one of the inhibitors combined with 1% BME.

4. Taking pictures of cultures After culturing cells for 6 days (for the dose-response study) and 3 days (for the inhibitor studies), pictures of cultures were taken at 5x, 10x, and 40x, and cell viability was determined using Trypan blue dye.

5. Glucose clearance from the medium After culturing cells for a required number of days, culture was centrifuged, medium was obtained and remaining glucose in the DMEM medium was measured using a glucose monitoring system (Biosensor Sciences).

6. Western blot After culturing cells for a required number of days, culture was centrifuged and cells were collected, protein was extracted using RIPA buffer and proteins were separated on 10% SDS-PAGE gel, transferred to PVDF membrane, and probed with the indicated primary antibodies. Western blot was done with sample size of one for three times.

7. Statistical significance Repeated t-tests were used to determine statistical significance for all experiments. However, with anticipated increased number of observations, we plan to use multiple comparison ANOVA.

Results

Figure 2. MCF-7 Cell Culture

1. Bitter melon extract (BME) dose-dependently exerts cytotoxic effect on MCF-7 cells.
2. BME decreases glucose clearance from the medium possibly due to less number of viable cells remaining in BME treated cultures.
3. ROK inhibitor, H-1152, seems to increase viability of MCF-7 cells (non-significant).
4. Protein kinase C does not affect viability of MCF-7 cells.

Figure 3. BME dose-dependently decreases number of MCF-7 cells adhered to the culture flasks.

Figure 4. Glucose measurement (N=4)

1. Bitter melon extract (BME) dose-dependently decreases glucose from the culture medium.

Figure 5. Western Blot MCF-7 Cell Viability (N=3)

Conclusions

1. Bitter melon extract (BME) dose-dependently exerts cytotoxic effect on MCF-7 cells.
2. BME decreases glucose clearance from the medium possibly due to less number of viable cells remaining in BME treated cultures.
3. ROK inhibitor, H-1152, increases viability of MCF-7 cells (non-significant).
4. Protein kinase C does not affect viability of MCF-7 cells.

Future directions

1. Measure the changes in phosphorylation of ROK target protein, MYPT1, and that of PKC target protein, CPI-17, with bitter melon extract treatment.
2. Measure the change in phosphorylation of Myosin Light Chain with bitter melon extract treatment.
3. Measure cytotoxicity induced by bitter melon extract using Lactate Dehydrogenase (LDH) and MTT assays which are more specific than Trypan Blue exclusion assays that we performed.