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

Heeyun Choi  
_Marian University - Indianapolis_

Bhupal Bhetwal Ph.D  
_Marian University - Indianapolis_

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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 biotin, 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]. ROK phosphorylates MYPT1 [Myosin targeting subunit of myosin light chain kinase (MLCK)] and inhibits it. Thus, ROK and PKC both inhibit MLCP activity favoring balance of 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 inhibitor 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)

Freshly harvested bitter melons were purchased from an Asian grocery store. They were washed and ground in a juice extractor. The juice extract was filtered at 500 RPM for 1 hour, and then sterilized. BME was 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 in the following conditions: 0% BME, 1% BM medium, 2%, 5%, and 10% of BME (v/v).

3. Testing roles of Rho kinase (ROK) and protein kinase C (PKC) inhibitor

To study whether PKC or ROK play any role in mediating BME’s effects, 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)].

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 magnification.

5. Glucose clearance from the medium

After culturing cells for a required number of days, glucose in the medium was measured by glucose meter (Bio Reactor Sciences GF-100) due to less number of viable cells remaining in BME treated cultures.

6. Western Blot

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

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, seems to increase 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.