Exploratory study for anti-metastatic effect of Curcumin and its analogues (PGV-0 and PGV-1) in breast cancer model

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Abstract

In this study, we tested the efficacy of curcumin and its analogues (PGV-0 and PGV-1) against breast cancer. We first tested effects of those curcumin analogues on viability of 4T1 cells as well as activity of NF-kB in 4T1 cells which is well-known target of anti -cancer effect of curcumin. We further tested the anti-metastatic effect of PGV-0 and PGV-1 in the experimental metastasis model of 4T1 breast cancer.

Backgrounds

Chemoresistance of breast cancer to doxorubicin is mediated mainly through activation of NF-kB and over expression of HER2. We previously reported Curcumin and its analogues (PGV-0 and PGV-1) exert cytotoxic effects on T47D breast cancer cells. Suppression of NF-kB activation is suggested to contribute to this activity. We further demonstrated the effects of curcumin, PGV-0, and PGV-1 singly and in combination with doxorubicin on MCF-7/Dox cells featuring over-expression of HER2. In MTT assays, curcumin, PGV-0, and PGV-1 showed cytotoxicity effects against MCF-7/Dox with IC50 values of 80 μM, 21 μM, and 82 μM respectively. These compounds increased MCF-7/Dox sensitivity to doxorubicin. Cell cycle distribution analysis exhibited that the combination of curcumin and its analogues with Dox increased sub G-1 cell populations. Curcumin and PGV-1 but not PGV-0 decreased localization of p65 into the nucleus induced by Dox, indicating that activation of NF- kB was inhibited. Molecular docking of curcumin, PGV-0, and PGV-1 demonstrated high affinity to HER2 at ATP binding site. This interaction was directly comparable with those of ATP and lapatinib. These findings suggested that curcumin, PGV-0 and PGV-1 enhance the Dox cytotoxicity to MCF-7 cells through inhibition of HER2 activity and NF-kB activation, therefore attractive targets for testing in vivo efficacy.

Materials and Methods

Compounds

Curcumin, PGV-0, and PGV-1 were dissolved in Dimethyl Sulfoxide (DMSO) then diluted in cell culture medium before being applied.

Cells

4T1 cell was cultured in RPMI -1640 medium supplemented with 10% fetal bovine serum.

Cell viability analysis

Viability analysis was done using a WST-1 Cell Counting Kit . Cells ($2x10^4$/well) were seeded in a 96-well plate. After 24-h incubation, the cell were treated with compounds for 24 h, then 10 µl WST-1 reagent was added and incubated for another 2 hr (37°C, 5% CO_2) . The absorbance at 450nm/620nm was measured using a microplate reader.

NF-$_K$B luciferase reporter assay

4T1-NFkB-luc cells were placed at $5x10^4$ cells/well in a 96-well plate. After 24 h incubation, the cells were treated with compounds, then 20 µl of luciferin (900 µg/ml) was added and the plates were incubated and checked the luciferase activity in every 2, 4, and 6 h.

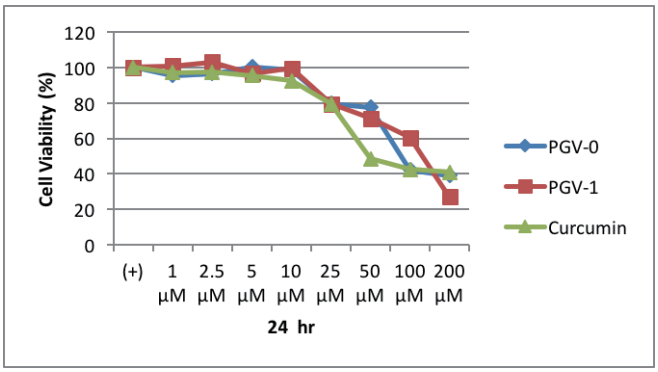
Exper imental lung metastasis model.

Female BALB/c mice (8 weeks) . 4T1-luc cells were inoculated intravenously (i . v, 5x105) with or without pre- treatment with curcumin or its analogues (24h,25µM) . After 4 days of tumor inoculation, the lungs were removed and incubated with D-luciferin to measure luminescence using IVIS.

Results and Discussion

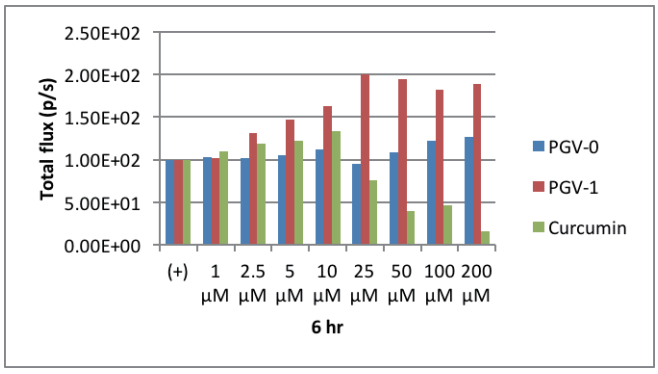
Effect of curcumin and its analogues on 4T1 breast cancer cell

We first determined an effect of curcumin or its analogues PGV-0, and PGV-1 on 4T1 cell viability. As shown in the figure below, all compounds showed similar effect on 4T1 cell viability in 24 hr co-culture.



Effect of curcumin and its analogues on NF-$_K$B activity in 4T1 breast cancer cell

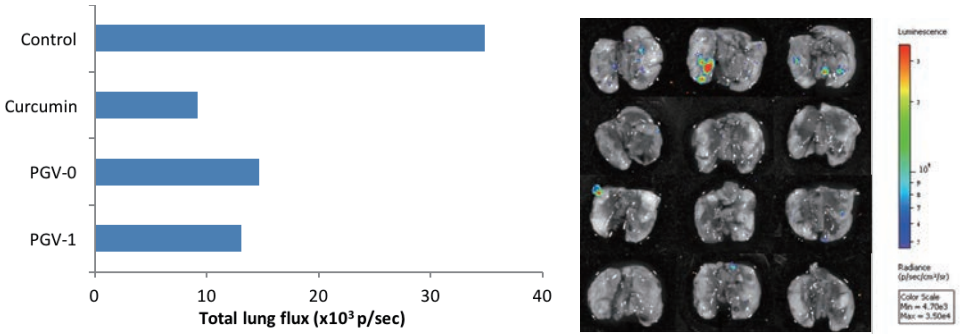
We next tested an effect of curcumin or its analogues PGV-0 and PGV-1 on NF-$_K$B activity in 4T1 breast cancer cell. While curcumin showed strong inhibitory effect on NF-$_K$B activity after 6hr co-culture, its analogues PGV-0 and PGV-1 did not affect NF-$_K$B activity in 4T1 breast cancer cells as shown in the figure below. These data suggest that both PGV-0 and PGV-1 have similar anti-cancer potential to crucumin, the mechanism of action should be distinct from curcumin.



Effect of curcumin and its analogues on metastatic potential of 4T1 breast cancer cell in vivo

We then examined an effect of curcumin or its analogues PGV-0 and PGV-1 on metastatic activity of

4T1 breast cancer cell in vivo. As shown in the figure below, all compounds showed significant effect on 4T1 cell lung metastasis treated with 24 hr prior to intravenous injection as shown in the figure below. These data suggest that both PGV-0 and PGV-1 have a promising potential as anti-cancer and anti-metastatic drug with a distinct mechanism to its original curcumin compound.



References

Meiyanto E, Putri DD, Susidarti RA, Murwanti R, Sardjiman, Fitriasari A, Husnaa U, Purnomo H, Kawaichi M.

Curcumin and its analogues (PGV-0 and PGV-1) enhance sensitivity of resistant MCF-7 cells to doxorubicin through inhibition of HER2 and NF-kB activation. Asian Pac J Cancer Prev. 2014;;15(1) :179-84.