Strigolactone analogs act as new anti-cancer agents in inhibition of breast cancer in xenograft model

This is a pre print version of the following article:

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/1530847 since 2015-12-02T15:17:00Z

Published version:
DOI:10.1080/15384047.2015.1070982

Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)
Strigolactone analogs act as new anti-cancer agents in inhibition of breast cancer in xenograft model

Elina Mayzlish-Gati1, Dana Laufzer1, Christopher F Grivas1, Julia Shalnoff2, Amiram Sananes2, Ariel Bier3, Shani Ben-Harosh4, Eduard Belousov1, Michael D Johnston2,2, Emma Arturko1, Oshrat Levy4, Oli Gelis1, Cristina Priado1, Isam Khalil1, Mark Pins1, Ronit Yarden5,6, Yoram Kapulkin1, and Hinatit Kohli1

1Institute of Plant Sciences, ARO, Volcani Center, Bet Dagan, Israel; 2Faculty of Engineering Sciences; The Anser and Sheila Goldstein-Goren Department of Botany and Plant Biology; Ben-Gurion University of the Negev Beer-Sheva, Israel; 3Department of Human Science, SUNY College at Brockport, Brockport, New York, USA; 4Department of Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada; 5Department of Microbiology, Weizmann Institute of Science, Woes, Israel; 6Department of Plant Sciences, Volcani Center, Bet Dagan, Israel

Keywords: breast cancer, cell motility, microtubule, plant hormone, strigolactone, xenografts.

Abbreviations: SLs, strigolactones.

Strigolactones (SLs) are a novel class of plant hormones. Previously, we found that analogs of SLs induce growth arrest and apoptosis in breast cancer cell lines. These compounds also inhibited the growth of breast cancer stem cell enriched-mammospheres with increased potency. Furthermore, strigolactone analogs inhibited growth and survival of colon, lung, prostate, melanoma, osteosarcoma and leukemia cancer cell lines. To further examine the anti-cancer activity of SLs in vivo, we have examined their effects on growth and viability of MDA-MB-231 tumor xenografts model either alone or in combination with paclitaxel. We show that strigolactones act as new anti-cancer agents in inhibition of breast cancer in xenograft model. In addition we show that SLs affect the integrity of the microtubule network and therefore may inhibit the migratory phenotype of the highly invasive breast cancer cell lines that were examined.

Introduction

Acquired or pre-existing resistance to therapy is a major contributing factor for cancer treatment failure. Current hormonal or chemotherapy modalities often result in initial favorable response but ultimately, poor survival, adverse effects and resistance lead to the unsuccessful elimination of tumors cells and to local or distant disease relapse. Metastatic disease is the main cause of cancer-related death.

Therefore, there is an ever-increasing need for development of safe drugs and novel therapeutic strategies that target both the highly proliferating cells as well as the slow-growing cancer cells in an irreversible manner without harming the normal cells.

Multiple plant hormones (phytohormones) including Cytokinin, Methyl Jasmonate and Brassinosteroids are known as effective anti-cancer agents. Strigolactones (SLs) are a novel class of plant hormones1 that are produced by a wide variety of plant species.2 A single plant species may produce several different forms of SLs but variation in combination of types and quantities of the different SLs may exist among the different members of a species. The SL structure consists of an ABC ring system connected via an enol ether bridge to a benzoate D ring.3 Currently about 17 natural SLs were identified and more than 35 synthetic analogs have been synthesized and used in plant studies.

Previously, we have shown that synthetic SL analogs induce growth arrest and apoptosis in breast cancer cell lines. We have shown that these compounds also inhibit the growth of breast cancer stem cell enriched-mammospheres with increased potency (Supplementary Table 1). The synthetic analogs, MEB55 and ST362, used in the previous and the current studies have an indole-based structure with a classical enol ether bridge connecting the C and D ring. The compounds are then further functionalized on the A ring with a dihydroxy ring in MEB55 and dioxanohydroxy in ST362 respectively. MEB55 is used as a racemic mixture (they differ for the configuration of C-2 of the D ring) whereas ST362 is a diastereomeric mixture (an additional stereo center is present on the C ring).

SL analogs treatment of cancer cell lines is associated with down-regulation of cyclin B1, which was partially repressed in the presence of prosurvival inhibitors and upon SL removal. In addition, SL analogs activate the stress activated MAPKs, PAR and JNK2 and inhibit the activity of the survival factors ERK1/2 and AKT.4 SL analogs also inhibit growth and survival of colon, lung, prostate, melanoma, osteosarcoma and leukemia cancer cell lines.5 The treatments with SLs result in the induction of G2 arrest, increased apoptosis, and loss of cell viability. It was suggested that SL analogs affect different cell types by similar mechanisms, positioning them as novel broad-spectrum anti-cancer agents.6

To further characterize the anti-cancer activity of SL analogs, we have examined their effects alone and in combination with paclitaxel on growth of the breast cancer cell line, MDA-MB-231, in culture and as xenograft model. The results substantiated SLs activity as anti-cancer agents of MDA-MB-231 breast cancer tumors. A further insight into the mechanism of SLs activity suggests that like paclitaxel, treatment with SLs leads to an effect on the integrity of the microtubule network and to inhibition of cell migration.

Results

MEB55 has low toxicity in mice

MEB55 was administered to mice at 12.5, 25, 50, 100 and 150 mg/kg. No differences were observed in mice body weight (BW) at all concentrations tested by either 8 or 14 d after the beginning of the treatment relative to the untreated controls (Fig. 1A). Liver sections stained with H&E indicated no histological changes up to 100 mg/kg while some abnormalities were observed at 150 mg/kg (Fig. 1B). Apoptotic cells in liver sections were apparent from 50 mg/kg treatment (Fig. 1C) and an increase in macrophage presence in the liver sections was detected in mice treated with 50 mg/kg of MEB55 (Fig. 1D).

No substantial toxic effects were found in mice treated with MEB55 at a dose of 25 mg/kg. Therefore, this dose was chosen to be tested for efficacy against MDA-MB-231 xenografts in mice. Notably, since MEB55 and ST362 are similar in structure and have both an indole based structure with a classical enol ether bridge connecting the C and D ring, an escalation dose was done for ST362 since results are expected to be similar.

MEB55 and ST362 inhibit breast cancer tumor growth in mice

Mice implanted with xenografts of MDA-MB-231 were examined for the effect of MEB55 on solid tumors. MEB55 (25 mg/kg) treatments led to reduction in tumor volume and to reduced tumor growth rate (Fig. 2). The post hoc analysis reveals that there are statistically significant differences between the tumor sizes in control and treatment (MEB55) groups at time points of 6, 11, 15, 18, 21, 24 d post injection (dpi) (Fig. 2A, Supplementary Tables 2-4). The interaction of treatment and time is statistically significant (p = 0.0017).

Similar results of reduction in tumor growth rate were also obtained for ST362 (25 mg/kg, Supplementary Fig. 1). Tumor growth rate of ST362 treated mice was significantly lower than tumor growth rate in control mice, and was similar to that of paclitaxel treated mice (Supplementary Fig. 1). At the end of the experiment, tumor weight of mice treated with MEB55 (25 mg/kg) and ST362 was reduced by 47%, 49% and 68% respectively, compared to untreated control. BW of treated mice was not significantly affected by either MEB55 or ST362 treatment (Supplementary Fig. 2 and data not shown, respectively).

MEB55 has an additive effect to that of paclitaxel in inhibition of growth and survival of breast cancer cell line

The effect of MEB55 in combination with paclitaxel was examined on the viability of MDA-MB-231 breast cancer cell line in culture. Dose–effect curves were determined for each of the compounds and for concurrent treatments of
MEB55 and paclitaxel (Fig. 3). For dose response assays, data points were connected by nonlinear regression lines of the sigmoidal dose-response relationship. GraphPad Prism (version 6 for windows, GraphPad software Inc., San Diego, USA) was employed to produce dose-response curves and IC50 doses for SLs and paclitaxel by performing nonlinear regression analysis. In each case, the upper limit was normalized to cell viability associated with treatment with the single, fixed-dose drug.

Addition of paclitaxel to MDA-MB-231 cells resulted in a sigmoidal concentration-dependent reduction in cell viability, with an IC50 of 16.87 nM (Fig. 3A). In the presence of 7.5 µM MEB55, MDA-MB-231 cells were sensitized as paclitaxel by 2.4 fold, i.e., IC50 of paclitaxel was 16.87 nM or 7 nM in the absence or presence of 7.5 µM MEB55, respectively (Fig. 3A). The enhanced sensitivity of MDA-MB-231 cells was noted only when cells were treated with paclitaxel at low concentrations (up to 25 nM after which levels of paclitaxel were too toxic to observe any additive effect).

Addition of MEB55 to MDA-MB-231 cells resulted in a sigmoidal concentration-dependent reduction in cell viability, with an IC50 of 5.8 µM (Fig. 3B). Sensitivity of MDA-MB-231 cells to MEB55 was enhanced 2 fold when cells were co-treated with 10 nM paclitaxel, i.e., IC50 of MEB55 was 5.8 µM or 2.4 µM in the absence or presence of 10 nM paclitaxel, respectively (Fig. 3B). The additive effect of 10 nM paclitaxel was apparent at all MEB55 treated concentrations, up to 25 µM, which was the highest MEB55 concentration used. At this high concentration paclitaxel had no significant additive effect on MEB55 treatment. Together, these results suggest an additive effect of paclitaxel and MEB55 on growth inhibition of MDA-MB-231 cancer cell growth.

Both MEB55 and paclitaxel act in inhibition of breast cancer tumor growth in animal model. Since MEB55 and paclitaxel showed an additive inhibitory effect on breast cancer cell line growth, we examined the combination of MEB55 and paclitaxel treatments on xenografts of breast cancer in mice. Mice were treated with either a low dose of paclitaxel (7.5 mg/kg) or a high dose of paclitaxel (15 mg/kg). As expected, paclitaxel at a high dose significantly inhibited the growth of MDA-MB-231 xenograft tumors. MEB55 by itself or a lower dose of paclitaxel (7.5 mg/kg) were not as effective in retarding tumor growth. Concurrent administration of MEB55 and the low-dose of paclitaxel reduced to some extent, but not significantly, tumor volume compared to treatment with MEB55 only. Similarly, concurrent administration of MEB55 and the low-dose of paclitaxel resulted in tumor volume decreases as compared to treatment with paclitaxel alone.

Discussion

We have previously shown that synthetic retinol analogs, including MEB55 and ST362, inhibit breast cancer cell growth and survival. In the present study we show that these analogs are effective in inhibiting growth of MDA-MB-231 xenograft tumors in animal model. Moreover, MEB55 at an effective concentration of 25 mg/kg caused only low level of toxicity, suggesting that it has the potential to be developed as an anti-cancer agent.

Furthermore, we have found that the concurrent administration of MEB55 and paclitaxel lead to an additive growth inhibition of MDA-MB-231 cultured cells. The additive effect was apparent only when the compounds were administered at relatively low concentrations. At higher concentrations of MEB55 or paclitaxel, no additive effects were apparent between the 2 compounds in cultured cells. The additive effect obtained with
concurrent administration of ME355 and pacitaxel suggest that the 2 compounds affect cell proliferation by a similar mechanism. Therefore, at higher concentrations, the maximal effect of each compound may mask the effect of the other compound. However, in subsequent experiments, treatment with ME355 alone did not improve significantly tumor inhibition vs. ME355 and pacitaxel combined treatment. Therefore, it cannot be concluded that ME355 enhances the efficacy of pacitaxel on solid tumor growth.

We found that ME355 and ST362 affect the integrity of the microtubule network. Shorter hours of treatment microtubule bundles are formed in the cytosol and around the cell nucleus. Interestingly, microtubules are the main target of paclitaxel or in cells. More specifically, paclitaxel was shown to be successfully targeted to class I and II β-tubulin, and lead to rearrangement, depolymerization, and inducing a more GTP-like configuration in the microtubule protofilaments, leading to formation of microtubule bundles (Fig. 5A and, e.g., 8). However, it is unknown whether SLA is a microtubule targeting agent.

Alterations in microtubule dynamics can influence cell migration via modulation of their subcellular pathways (15). Therefore, we have examined the effect of ME355 on cell migration. In accordance with its effect on microtubules, ME355 reduced the migration ability of 2 breast cancer cell lines (MDA-MB-231 and MDA-MB-468). Since cell migration was shown to be involved in metastasis formation (e.g., 16), these results may indicate that ME355 may lead to reduced metastasis.

In summary, an analog of ME355 and ST362 inhibit MDA-MB-231 tumor development in xenograft model. It would be of interest to determine whether SLA targets microtubules directly. ME355 relatively low toxicity and high efficacy suggest its usage as an anti-cancer agent to promote anti-cancer activity while reducing chemotherapy toxicity.

Materials and Methods

Cell lines

Cells were grown at 37°C in a humidified 5% CO2:95% air atmosphere. All tissue culture media and serum were purchased from Biological Industries Ltd. Israel, unless otherwise indicated. MDA-MB-231, ATCC, Manassas, VA) were maintained in DMEM supplemented with 10% FCS, 1% Penicillin-Streptomycin Solution, and 1% L-glutamine. Visible cells were counted under a microscope by trypan blue (Sigma-Aldrich) exclusion.

Cell proliferation assay

Cells were seeded into a 96 well plate at 2,500 cells per well in triplicates in normal growing media. On the following day, the media was replaced with phenol red-free DMEM supplemented with 10% FBS (Foetal Bovine Serum) and 1% Penicillin-Streptomycin Solution. Cells were incubated overnight at 37°C in a humidified 5% CO2:95% air atmosphere. Absorbance was recorded at 570 nm with 10% FBS to determine the time of cell confluence, at 24 h in the presence of growth medium containing 1% FBS (to eliminate the possibility of cells filling the scratch by proliferation) and/or the indicated concentrations of ME355 or with DMEM as vehicle control, and images of the same marked points in each well were taken again. Finally, images taken at time 0 and 24 h were compared. Cell survival was measured in each image using “IMAGEJ” software. % Recovered area, measured from the images, was calculated as [ % cell free area in treatment - 24 h cell free area in untreated - 24 h cell free area in untreated] x 100. At least 2 independent experiments were done for each treatment; in each experiment at least 3 technical replicates were performed.

Cell migration studies

Cell motility assay was conducted as described in Liang et al. (17), with several modifications. 1 x 10^4 or 1 x 10^5 MDA-MB-231 or MDA-MB-435 cells were seeded in each well of a 24-well plate, in volume of 1 ml of growth medium. The cells were then spread throughout the well and allowed to adhere for 24 h. On the following day, the cell culture medium was then removed and replaced with a medium containing ME355 treatment in 2 concentrations, or medium only for untreated control. In addition, treatment was treated with DMEM as vehicle control. Incubation was for another 24 h. Next, the medium was removed and the cells monolayer was scraped in a straight line using a 200 µl pipette tip to create a scratch. Debris were removed, and the edges of the scratch were smoothed by washing the cells once with 1 ml of growth medium. Images of 3 marked points along the scratch in each well were taken with a phase-contrast microscope for time 0. Cells were incubated for additional 24 h in the presence of growth medium containing 1% FBS (to eliminate the possibility of cells filling the scratch by proliferation) and/or the indicated concentrations of ME355 or with DMEM as vehicle control, and images of the same marked points in each well were taken again. Finally, images taken at time 0 and 24 h were compared. Cell survival was measured in each image using “IMAGEJ” software. % Recovered area, measured from the images, was calculated as [ % cell free area in treatment - 24 h cell free area in untreated - 24 h cell free area in untreated] x 100. At least 2 independent experiments were done for each treatment; in each experiment at least 3 technical replicates were performed.

Streptolysin analogs and ME355 were dissolved in DMSO (D2560; Sigma) at stock concentrations of 10 mM. Cells were treated at the indicated doses by diluting the analog to the required highest concentration in the appropriate culture medium. Serial dilutions were performed for subsequent lower concentrations. At least 2 independent experiments were done for each treatment; in each experiment at least 3 technical replicates were performed.

Paclitaxel treatments

Paclitaxel (T7402; Sigma) was solubilized in DMSO at stock concentration of 10 mM. Cells were treated at the indicated doses by dilution to the required highest concentration in the appropriate culture medium. Serial dilutions in culture medium were performed for subsequent lower concentrations. At least 2 independent experiments were done for each treatment; in each experiment at least 3 technical replicates were performed.

Animal model experiments

Determination of streptolysin analogs toxicity

Mice Hae/Cre/CD4D17 (Blain, Israel) were housed at a 12-hour light/12-hour dark cycle were treated with different concentrations of ME355, one of the most potent SL analog, in concentrations ranging from 25, 50, 100 and 150 mg/kg. Each group was set as vehicle control (n = 5 mice/treatment). Mice were injected intraperitoneal (IP) twice a week with the ME355 solution, dissolved with DMSO:Chromophor 2:1.14 dilution with double distilled water to the required concentration. Body weight (BW) was determined at times 0 and 16 day post injection (DPI) of ME355. At the end of the experiments, at 14 DPI, liver, from control and treated mice were harvested, fixed with formalin and sectioned, as described below. All procedures were conducted in accordance with the institutional (IL-1-62-2012, IL-88-12-2012, IL-49014) and national guidelines.

Determination of streptolysin analogs and pacitaxel effect on tumor growth

Mice BALB/c/OlaHsd-1 weeks were implanted subcutaneous with 2 x 10^6 MDA-MB-231 cells. The tumor were allowed to grow until they reach an average of 35 mm3 (about 2 weeks) and then mice were randomly assigned to the following groups (n = 8 mice/treatment): Experiment 1 consisted of the following treatments: (1) ME355 (25 mg/kg); (2) control vehicle. Experiment 2 consisted of the following treatments: (1) ST362 (25 mg/kg); (2) paclitaxel (20 mg/kg); (3) vehicle control. Experiment 3 consisted of the following treatments: (1) paclitaxel (7.5 mg/kg); (2) paclitaxel (15 mg/kg); (3) ME355 (25 mg/kg); (4) ME355 (25 mg/kg) and paclitaxel (7.5 mg/kg); (5) ME355 (25 mg/kg) and paclitaxel (7.5 mg/kg); (6) ME355 (25 mg/kg) and paclitaxel (7.5 mg/kg); (7) ME355 (25 mg/kg) and paclitaxel (7.5 mg/kg). Administration was by IP twice a week for 3 weeks and mice survival and tumor volume were recorded. At the end of the experiments BW was determined prior to tumor harvest. Tumors volume was calculated as V = (π/6) x (width x length x length).
Histological examination

Liver biopsies were fixed overnight in 4% paraformaldehyde in PBS at 4°C, embedded in paraffin 5-μm sections were pre-
pared and stained with hematoxylin and eosin (H&E). For mac-
roraphes, determination, immunohistochemistry was performed
using polyclonal anti-macrophage antibodies, ICI 184,442, Acris,
Hidenhauern, Germany) at 1:250 dilution. To evaluate the
number apoptotic cells in the liver sections the DeadEnd
Fluorometric TUNEL system kit (Promega) was used. This kit
detects fragmented DNA of apoptotic cells.

Statistical Analyses

Results are presented as mean ± SE of replicate analyses and
are either representative of or inclusive of at least 2 independent
experiments. Means of replicates were subjected to statistical
analysis by Student’s t-tests (P < 0.05), using the JMP statistical
package and regarded as being significant when P < 0.05 (†).
GraphPad Prism (version 6 for windows, GraphPad software
Inc., San Diego, USA) was employed to produce dose-response
curve and IC₅₀ doses for SLs and paryltxyl by performing non-
linear regression analysis. Tumor volume (0.5 × small diameter2
× large diameter) was calculated and significant differences
were analyzed by Wald test of tumor size change at different time
points for mice in control and treatment groups (P < 0.05).

Disclosures of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Lin, L., Tan XZ. Cross-talk between physiologic
org/10.1021/cr100309k.
2. Unsworth M, Stevens A, Yoshida S, Ayman K, Arter
J, Anderson T, Askari MI, Balentine T, Bambir R, Berost
K, Takahashi K, et al. Methods of destaining by the propylene glycol formalin. Anatom Rec 2004; 278:111. PMID:
3. Gorroz-Rubio V, Acosta S, Stewart PB, Pacheco P,
Díaz-Díaz P, Lutter T, Mirejovsky K, Davidon
4. Sei X, Yoshiba Y, Takahashi K. The Histological Scoring
5. Zawadzka A, Nijazi SK, Chakravarty TV, Bezio-
mmann H. New endogenous estrogens: structure-
activity relationship and role of exogenous in generating
1055/s-0035-1499006.
6. Plunkett C, Chuchley TF, Talbott S, Bermea P, Nunez
M, Krieger D, Bena MD, Millman L. Non-pancreatic endocrine analysis of estrogen-producing endocrine and
biological functions to promote weight gain and increase blood. Eur J Organo Chem 2011; 2011:91-99. doi:
7. Plunkett C, Krieger D, Kugeloff S, Plunkett C, Yuend R.
Histological analysis of a novel class of physiologic estrogens that
while the growth and survival of breast cancer cells and老师的《生理学与病理学》方面的研究。

Acknowledgments

We thank Avner Levin, Moran Gelber and Peter Johnson for
technical assistance, and Sheligla a. for providing estrogeneic
analogue.

Funding

This research was partially funded by ‘Kamim’ fund of The
Israel Ministry of Economy (IK and YK), The Chief Scientist
Fund of The Israeli Ministry of Agriculture (IK), Georgetown
SNHS research award (RIT) and Georgetown Undergraduate
Research Opportunities Program, GUROP, (CGT). The immu-
nofluorescence experiments were performed with advice and tech-
nical assistance of Microscopy and Imaging Shared Resource of the
Leventhal Comprehensive Cancer Center partially supported
by NIH/NCI grant P30-CA51008. Statistical analysis was pro-
vided by Dr. Hongkun Wang from the Georgetown Univeity
Biostatistics & Bioinformatics Shared Resource that is partially
supported by NIH/NCI grant P01-CA95100 and G5U/CCTS
grant UL1 TR000110.

Supplemental Material

Supplemental data for this article can be accessed on the
publisher’s website.

www.tandfonline.com

Cancer Biology & Therapy

7