Evaluation of anti-neoplastic acitivity of LAB-derived postbiotics as a potential tool to suport standard therapy in the most common types of cancer that affect women

International Doctoral School
Medical University of Lodz

Phd student: Joanna Wasiak[1],
Supervisors: Monika Witusik Perkowska[1], Janusz Szemraj[1]
[1] Department of Medical Biochemistry, Medical University of Lodz

Introduction

Recent advancements highlight the potential of probiotics as promising agents in breast cancer prophylaxis and therapy since about 20-30% of patients with ER-positive phenotype breast cancer are resistant to hormonal treatment with standard drug – tamoxifen (TAM), thus it's important to find new therapeutic solutions based on a synthetic or natural compounds. Postbiotics, as metabolic products of probiotic bacteria, were proven to have sufficient bioactivity to exert pro-health and anti-cancer effects, appointing them viable adjunctive agents for treatment and prevention of various neoplasms.

Materials and methods

Breast cancer cel line (MCF7) and normal fibroblast cell line (WI-38) were subjected to treatment with synthetic compounds - TAM and a new anti-cancer agent - ARA12 (an aziridine–hydrazide hydrazone derivative) and their combination with LAB-derived postbiotics obtained from *Lacticaseibacillus rhamnosus GG* and *Lactiplantibacillus plantarum 299v*. Post-treatment analyses included cell viability assay based on resazurin conversion measurement and flow cytometric detection of apoptosis/necrosis fractions by means of annexin V-FITC/PI staining. Cell cycle progression and proliferation were analyzed by PI-based flow cytometry and Ki-67 immunostaining.

Results

Postbiotic treatment caused reduction of neoplastic cells' viability, while the mixture of synthetic therapeutics with LAB metabolites induced a significant decrease in cells' survival in comparison to cells treated with a single agent. FACs results also demonstrated that bacterial metabolites have the ability to stimulate apoptosis in cancer cells. Additionally, LAB postbiotics showed selective antineoplastic effect with no negative impact on normal cells. Cell cycle analysis of MCF7 cells treated with LAB-derived PM and their combinations tested drugs shows the effect of postbiotics on the cell cycle, visible as a shift to S phase. LAB-PM combinations with synthetic compounds inhibit the cell cycle in the G0/G1 phase, enhancing the effect seen for TAM and ARA12 alone. Also immunofluorescence analysis of the proliferation marker Ki-67 showed the effect of LAB-derived PMs in reducing its expression to almost undetectable levels in cells treated with the synthetic compounds TAM and ARA12 in combination with postbiotics.

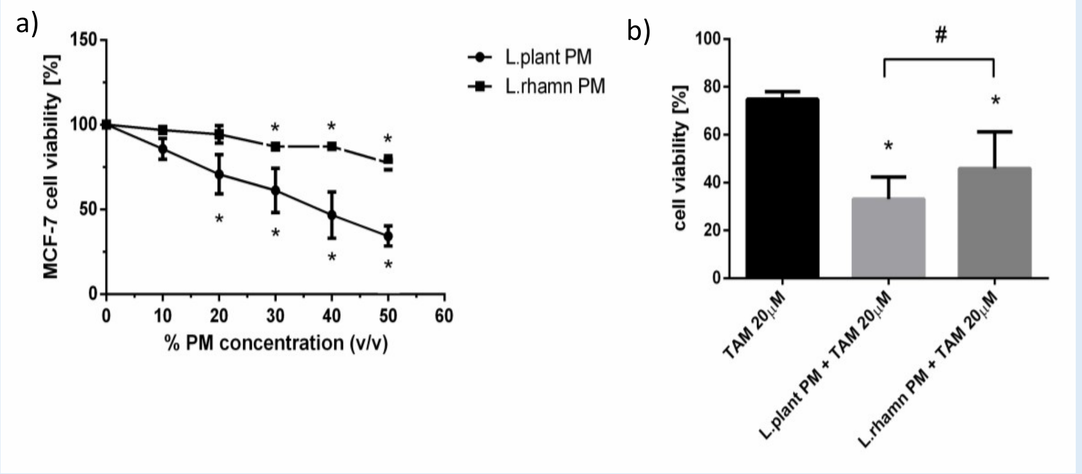

Fig.1. Response of breast cancer cells to LAB-derived postbiotics and their combinations with tamoxifen based on resazurine viability assay. a) Survival analysis of MCF7 after 72-h treatment with a range of PM concentrations obtained from tested LAB strains. b) Combinations of TAM (20 μM) with LAB-PM (30% v/v); $p<0.05$

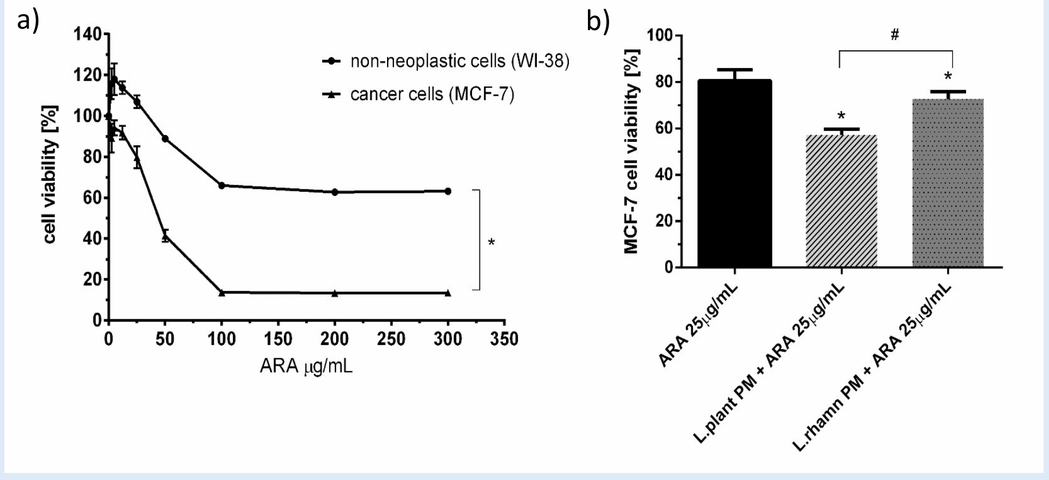

Fig.2. Response of breast cancer cells to new candidate drug - ARA12 and its combinations with LAB-derived postbiotics. a) Survival analysis of cancer cell line MCF7 and normal cell line WI-38 after 72-h treatment with a range of ARA12 concentrations b) Combinations of ARA12 (25 μg/mL) with LAB-PM (30% v/v); $p<0.05$

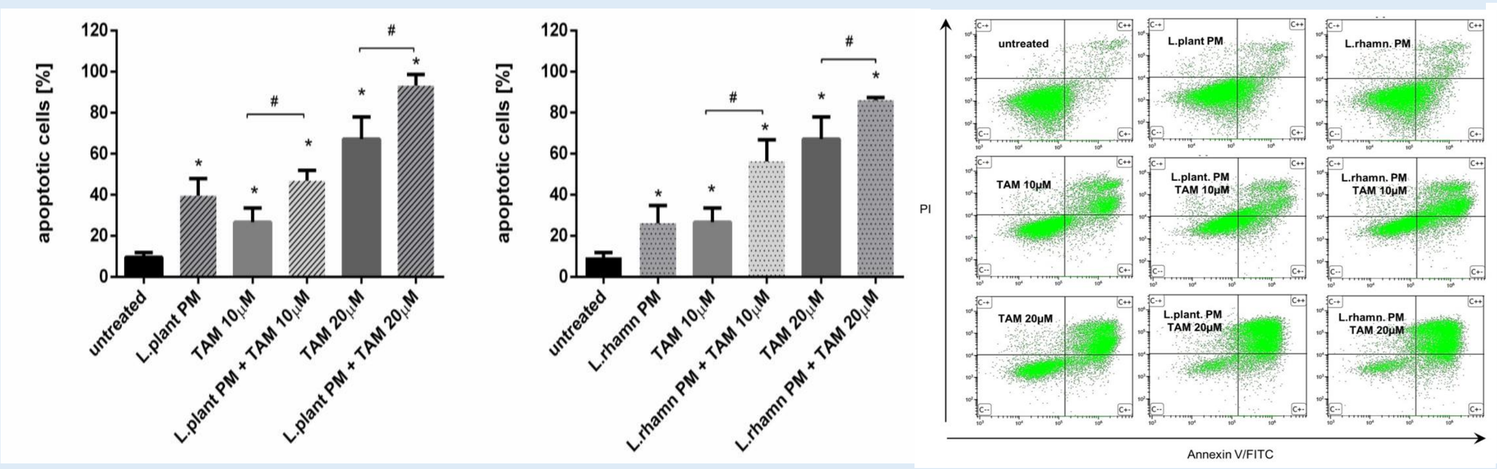

Fig.3. Results of cell viability analysis based on annexin V/PI assay performed for MCF-7 cells after 72h treatment with LAB-derived PMs and their combinations with TAM (10 and 20μM). FACs analysis presenting proapoptotic activity of LAB-PM improving anticancer potential of TAM; $p<0.05$

Fig.4. Results of cell viability analysis based on annexin V/PI assay performed for MCF-7 cells after 72h treatment with LAB-derived PMs and their combinations with ARA12. FACs results showing proapoptotic activity of ARA12 enhanced by LAB-PM; $p<0.05$

Conclusions

Results of our in vitro study suggest that postbiotics seems to be promising tool to increase the effectiveness of standard therapeutic - TAM and a new candidate drug - ARA12, showing their multipotential properties as natural adjunctive agents supporting anti-cancer treatment based on various synthetic drugs.

This research was funded by National Science Centre, Poland, grant number . 2022/47/O/NZ7/01089 (PRELUDIUM BIS).
The manuscript based on the presented results has been submitted to the Molecules journal (IF 4.6; MNiSW 140)