International Doctoral School
Medical University of Lodz

Presenting author:Pola Głowacka
Monika Witusik-Perkowska, Janusz Szemraj
Department of Medical Biochemistry, Medical Universiy of Lodz, 92-215 Lodz

Antineoplastic and cytoprotective properties of postbiotics as potential nutraceutics in adjunct therapy of central nervous system disorders

Thesis supervisor: Prof. dr hab. n.med. Janusz Szemraj
Thesis supporting supervisor: dr. hab. n. med. Monika Witusik-Perkowska

Background

Glioblastoma therapy is hindered by multiple processes which are often caused by applied therapeutics, such as cellular senescence. The neoplasm typically occurs in the elderly, and the treatment, both by chemical agents and irradiation, triggers premature cellular senescence induced by stress and is connected to tumor therapy escape mechanisms and often causes “chemo brain” that involve damage to normal cells and results in a significant cognitive decline. Up to date, glioblastoma remains practically incurable.

Postbiotics are metabolic products and macromolecules produced by probiotic bacteria, such as lactic acid bacteria (LAB). Certain ingredients of the LAB postculture mixture, e.g. short-chain fatty acids (SCFAs), lactic acid or indole metabolites and bacterial extracellular vesicles (BEVs) that pass the blood-brain barrier and exert direct influence on central nervous system cells. Postbiotics have been observed to cause a selective cytotoxic effect towards cancer cells while being able to protect normal cells from various stressors. This makes them appealing candidates for nutraceutics in CNS disorders adjunctive therapy, where they would protect normal cells from chemotherapeutics and sensitize cancerous cells at the same time.

Research aim and experimental tasks

The main objective was to analyze the antineoplastic potential of **postbiotics** and their interactions **with synthetic compounds** with antineoplastic activity on in vitro **glioblastoma models**, including patient-derived cell lines, as well as to assess postbiotics influence on **normal cells**, including normal CNS cells.

It was hypothesized that postbiotics derived from commercially available probiotic lactic acid bacteria strains, **Lacticaseibacillus rhamnosus** and **Lactiplantibacillus plantarum,** including **BEVs** isolated from the postculture mixture:

- exert antineoplastic effect towards multiple GB cell lines while showing no effect or positive one on normal cells,
- sensitize GB cells to currently applied chemotherapy (TMZ) or therapy conducted with novel compounds (ARA),
- sensitize GB cells to irradiation,
- show cytoprotective properties towards normal cells undergoing the same therapeutic conditions as GB cells.

Methods

Experimental tasks were performed using following methods:

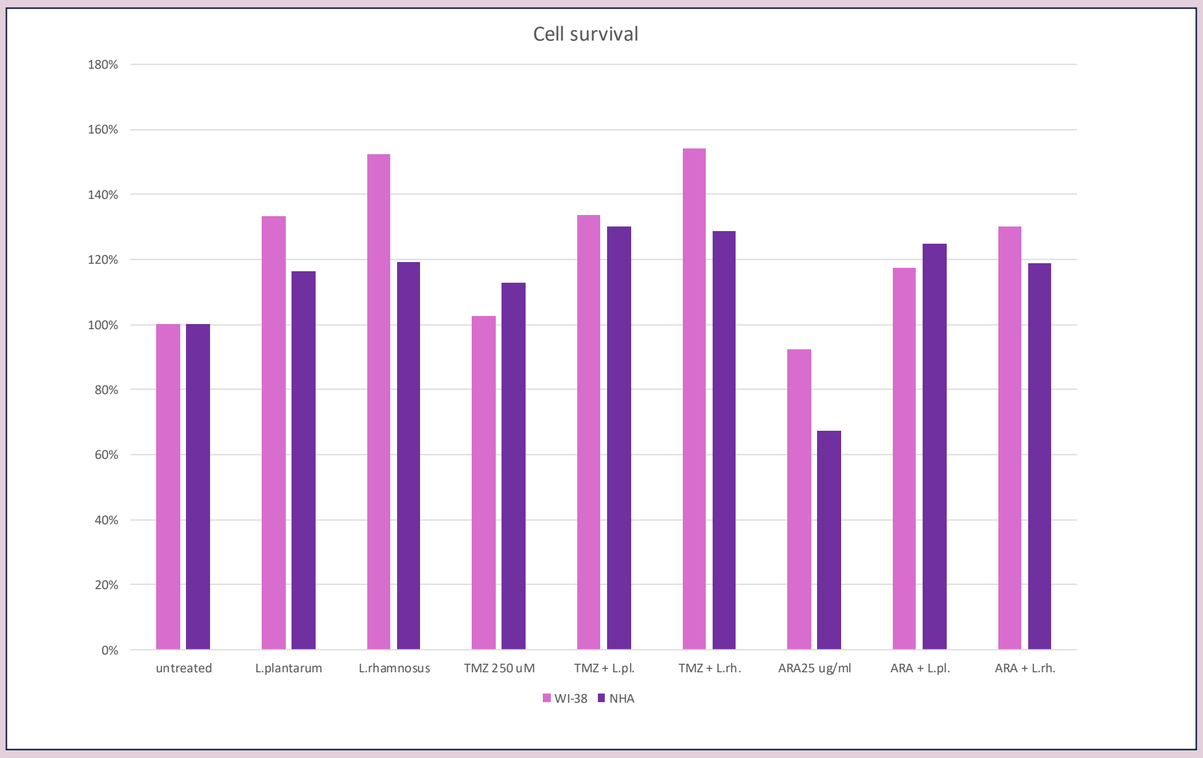
- Cell culture (U87MG. Patient-derived GB cell lines, normal cells: NHA, WI-38) and bacteria culture (*L. plantarum, L.rhamnosus*) performance, BEV isolation from bacteria postculture mixture;
- Cell viability assessment conducted on a 96-well plate with Presto Blue assay (based on fluorescence reading);
- Analysis of cell death processes, including necrosis and apoptosis - detection by flow cytometry of cells stained with annexin V labeled with FITC and propidium iodide;
- Cellular senescence analysis by senescence-associated beta-galactosidase staining and detection of caspase 3 activity by immunocytochemistry staining.

Results

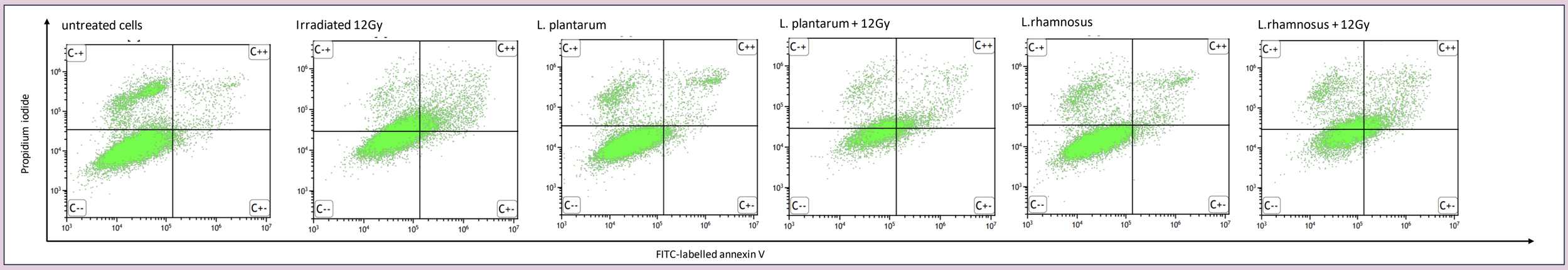
Cells viability and cell death analysis via flow cytometry (data expressed as mean±SD)

%	Untreated cells	L. plantarum	L. rhamnosus	TMZ	L. plantarum + TMZ	L. rhamnosus + TMZ	ARA	L. plantarum + ARA	L. rhamnosus + ARA
U87									
Viable cells	**89.06 ±3.27**	**69.03 ±4.81**	**79.90 ±4.22**	**70.94 ±3.39**	**52.82 ±8.11**	**73.45 ±2.10**	**79.96 ±3.14**	**51.72 ±1.66**	**55.19 ± 6.65**
Apoptosis	7.71 ±4.58	19.88 ±7.10	14.18 ±5.13	20.56 ±2.96	30.99 ±11.56	17.88 ±2.83	15.94 ±2.35	37.09 ±3.26	36.00 ±8.10
GB1									
Viable cells	**89.3 ±1.48**	**79.95 ±3.19**	**74.64 ±3.52**	**79.62 ±2.95**	**65.43 ±3.18**	**61.84 ±14.85**	**34.53 ±2.55**	**22.85 ± 5.26**	**24.26 ± 8.18**
Apoptosis	8.11 ±2.75	16.95 ±5.00	19.58 ±3.53	17.68 ±3.54	32.28 ±8.33	45.75 ±2.82	61.52 ± 3.16	72.84 ±8.82	73.83 ±10.43
GB2									
Viable cells	**80.65 ±3.15**	**67.52 ±3.93**	**67.70 ±3.27**	**70.02 ±1.29**	**71.15 ±3.35**	**72.82 ±0.89**		In progress.	
Apoptosis	16.42 ±1.98	29.15 ±4.05	28.31 ±3.45	26.42 ±1.32	23.51 ±2.75	22.90 ±1.01			
GB3									
Viable cells	**92.6 ± 1.87**	**89.92 ± 4.92**	**88.89 ± 7.40**	**87.70 ± 0.25**	**88.36 ±2.00**	**88.64 ±1.47**	**44.03 ±3.18**	**48.96 ±3.53**	**46.97 ±1.28**
Apoptosis	5.00 ± 1.32	6.20 ±2.06	7.48 ±4.37	9.62 ±5.28	8.99 ±5.04	9.20 ±5.12	48.46 ±4.88	44.04 ±3.46	46.55 ±1.18

Cell viability analysis via PrestoBlue Assay.

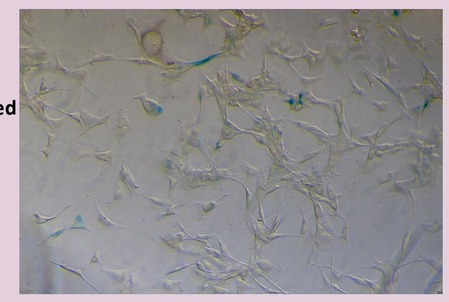


Cell viability and cell death analysis via flow cytometry for U87MG – representative results for irradiated cells and respective controls.

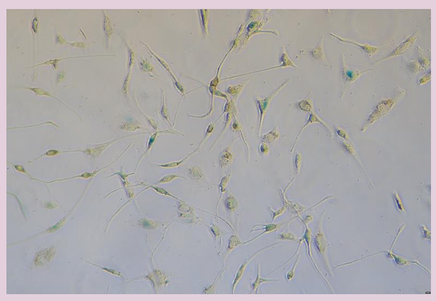


Cellular senescence staining via senescence associated beta-galactosidase detection performed for U87MG – representative results for irradiated cells and respective controls.

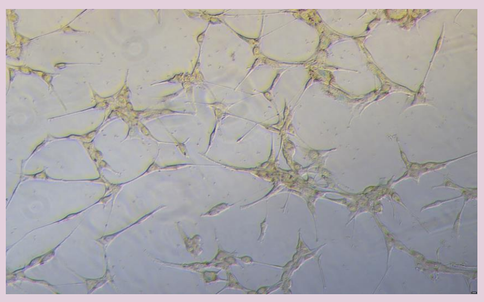
untreated cells



irradiated cells



L.plantarum



L.plantarum + radiation

Conclusions

- Postbiotics exert varied anticancer effect on different GB cell lines
- The scale of anticancer effect of combined therapy with postbiotics differs between GB models
- Postbiotics exert beneficial or no toxic effect on normal cells
- Postbiotics have cytoprotective properties toward normal cells subjected to anticancer therapy.

Therefore, postbiotics effect on cellular senescence and processes involved in both normal and pathological senescence-related neurodegeneration should be examined.