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Mechanisms of antimelanoma effect of oat β-glucan supported by electroporation

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ABSTRACT

There are still not specified mechanisms how beta-glucan molecules are transported into cells. Supposing, beta-glucan toxicity against tumor cells may be related to the overexpression of the transporter responsible for the transport of glucose molecules in the cells. In this case, glucans - polymers composed of glucose units are much more up-taken by tumor than normal cells. Increased GLUT1 (Glucose Transporter Type 1) expression has been demonstrated earlier in malignant melanomas. GLUT1 expression promotes glucose uptake and cell growth in that cells. Also, in human melanoma tissues a significant correlation between GLUT1 expression and mitotic activity was found.

The aim of the study was to verify if oat β-glucan (OβG) is delivered into cells by GLUT-1 membrane protein. To check it out we blocked GLUT1 transporters by an inhibitor WZB117 and then we investigated cells viability with and without reversible electroporation (EP).

The obtained results bring us to elucidate the mechanism of transport of the OβG into the cells is GLUT-1 dependent and moreover can be supported by EP method.

1. Introduction

In recent years the interest of the usage of plants for the production of pharmacological compounds has increased. Medicines derived from plants nowadays are becoming even more popular than standard chemotherapeutics. To compete with the growing synthetic drugs market, there is an urgency to scientifically validate more its usefulness in the medication [1]. The study of the metabolic drugs pathways is an essential and important part of the drug development process. During the drug evaluation the research of drug metabolism is of high importance especially when metabolites are pharmacologically active, toxic or when a drug is extensively metabolized [2].

Numerous studies have revealed that β-glucan has many therapeutic properties, including anticancer [3, 4]. It is non-cellulosic polymer of β-glucose, which is a glycoside in position β (1-3), (1-4) or β (1-6) [5, 6]. β-Glucans are carbohydrates found in the cell walls of yeast, fungi, algae, lichens, and plants such as oats or barley [6]. There are various sources from which β-glucan can be obtained. This enables to get a large number of formulations of similar or different properties [3]. Immunomodulatory and anti-cancer properties of β-glucans result mainly from their structure and degree of branching, but the mechanisms of anticancer activities of β-glucan seem to be multicomplex and still unclear [7–9]. There are still not specified mechanisms how β-glucan molecules are transported into cells. According to our hypotheses, β-glucan cytotoxicity against tumor cells may be related to the overexpression of the transporter responsible for the transport of glucose molecules to the cells. In this case, glucans - polymers composed of glucose units are much more up-taken by tumor than normal cells [4, 9].

Up to 90% of cancers demonstrate a phenotype of an increased glucose uptake and increased dependence on glucose as a source of energy and biosynthesis precursor for cell growth, while normal cells metabolize lipids, amino acids and glucose in a more balanced way [10, 11]. The increased glucose uptake by cancer cells is achieved primarily by upregulation of glucose transporters (GLUTs) [12]. The increased GLUT1 (Glucose Transporter Type 1) expression was demonstrated in malignant melanomas [13]. GLUT1 expression promotes glucose uptake and cell growth in that cells [14]. Also, in human melanoma tissues a significant correlation between GLUT1 expression and mitotic activity was found [13]. The overexpression of GLUT1 protein is related in poor prognosis in a wide range of solid tumors [15].

We investigated the possible involvement of GLUT1 in the transport of β-glucan to melanoma cells. In our experiments WZB117 was used.

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This is a small molecule, that effectively inhibits GLUT1 and cancer cell growth in vitro and in vivo [16, 17]. Previous studies showed that WZB117 impeded glucose transport in human red blood cells, in which GLUT1 is the only glucose transporter expressed [18]. This conclusively shows that WZB117 inhibits GLUT1. Different studies have shown that after the exposure to WZB117 cancer cells experienced an immediate reduction in glucose transport and it is the consequence of the decrease in the GLUT1 protein level after the WZB117 treatment [19].

For the verification how β-glucan is transported to cells, different variants of viability test were performed: after incubation with glucan, after blocking GLUT1, after incubation with glucan and simultaneous blocking of GLUT1 and finally after blocking GLUT1 and delivering glucan to cells by reversible electroporation.

2. Experimental

2.1. Cell culture

Two human malignant melanoma cell lines were used - Me45 and MeWo cell line. Me45 cell line (derived from a lymph node metastasis of skin melanoma in a 35-year-old male) was established in 1997 at the Radiobiology Department of the Center of Oncology in Gliwice, Poland, MeWo cell line was purchased from ATCC® (LGC Standards, Poland). The cells were grown as a monolayer in Dulbecco modified Eagle medium (DMEM, Sigma-Aldrich, USA) which was supplemented by 2 mM L-glutamine, 10% fetal bovine serum (FBS, Sigma-Aldrich, USA) and 50 μg/ml streptomycin (Sigma-Aldrich, USA) at 37 °C in 5% CO_2. Before every experiment cells were removed by 0.25% trypsin with 0.02% EDTA (Sigma-Aldrich, USA).

2.2. Chemicals

Oat β-glucan was courtesy of Mrs. J. Harasym from the University of Economics in Wroclaw and it was obtained due to the procedure described previously [20] with beta-glucanase inactivation during lipid removal step, alkaline extraction, protein removal in isoelectric point, solution neutralization to pH = 7.0 and beta-glucan precipitation with ethanol. The different concentrations of OβG were used to the studies (50 μg/ml, 100 μg/ml, 200 μg/ml, 400 μg/ml, 500 μg/ml).

The inhibitor WZB117 was purchased from Sigma-Aldrich (Poland). The following concentrations were used for experimental protocols: 2.5 μM, 5 μM, 10 μM, 15 μM, 20 μM, 25 μM.

2.3. Cellular viability - MTT assay

The viability of cells was determined by MTT assay (Sigma-Aldrich, USA) after experiments with different concentrations of beta-glucan (50–500 μg/ml), different concentrations of WZB117 (2.5 μM–25 μM) and after experiments where oat β-glucan, WZB117 or its combination was supported by electroporation. The MTT assay was used to the estimation of mitochondrial metabolic function through the measurement of mitochondrial dehydrogenase after 24 h incubation after experiments. For the experiment the cells were seeded into 96-well microculture plates at 1×10^4 cells/well. After incubation with selected concentrations of beta-glucan the experiments were realized according to the manufacture's protocol. The absorbance was determined using a multiwell scanning spectrophotometer at 570 nm (Enspire Perkin Elmer Multiplate reader, USA). Mitochondrial metabolic function was expressed as a percentage of viable treated cells in relation to untreated control cells.

2.4. Electroporation protocol

The electroporation was carried out using Gene Pulser Xcell total electroporation system (Bio-Rad, cat. number: 165-2660, purchased from Bio-Rad Poland). The electroporation protocol was selected according to the previous studies [19]. Cells in suspension were centrifuged for 5 min at 800 rpm and resuspended in the electroporation buffer with low electrical conductivity (10 mM phosphate, 1 mM $MgCl_2$, 250 mM sucrose, pH 7.4) with selected drug. After pulsation (eight 100 μs pulses, 400–1200 V/cm) cells were left for 10 min with an addition of DMEM at 37 °C, then centrifuged and seeded into culture 96-well plates for the MTT assay described in Section 2.3.

2.5. β-Glucan-WZB117-EP - combination therapy

Melanoma cells were trypsinized and suspended in electroporation buffer containing WZB117 in 25 μM concentration and β-glucan in concentrations: 50 μg/ml, 100 μg/ml, 200 μg/ml, 400 μg/ml, 500 μg/ml. Than cells were exposed to electroporation according protocol described in

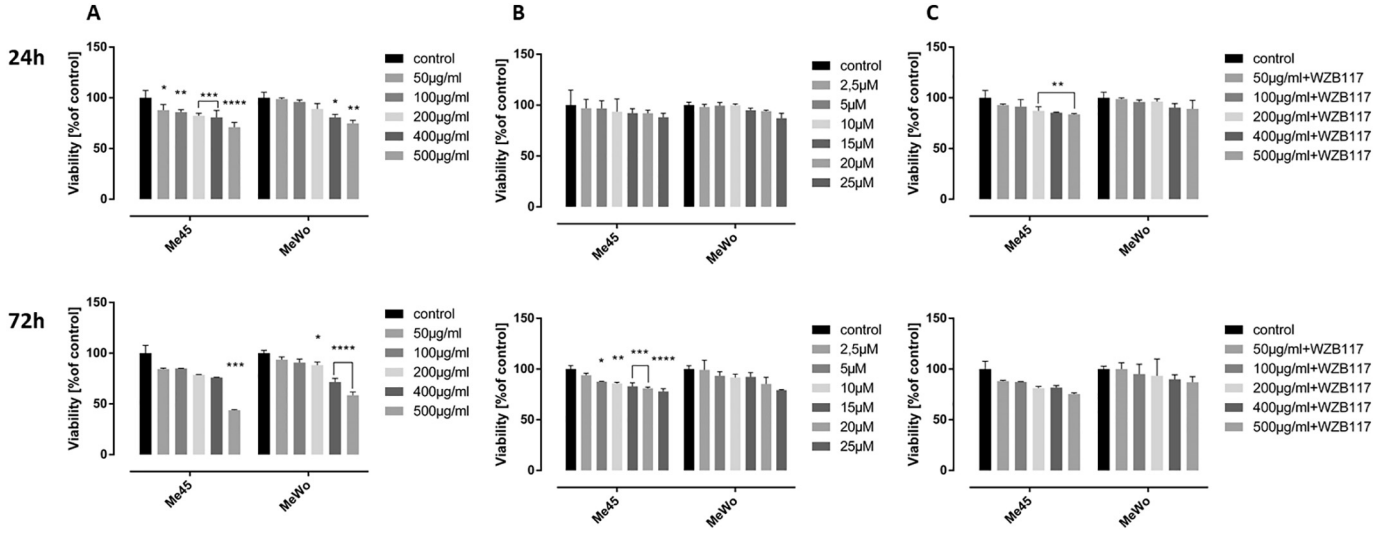

Fig. 1. viability of Me45 and MeWo cell line after 24 h and 72 h incubation following increasing concentrations of oat β-glucan (panel A), after incubation with the increasing concentrations of WZB117 (panel B), following increasing concentrations of oat β-glucan in combination with 25 μM WZB117 (panel C). Viability is expressed as the percentage of the control cells (cells without oat β-glucan). Error bars shown are means ±SD for n ≥ 3. *Statistically significant for $p \leq 0.05$.