

Research articles


Dichloroacetate, 2-Deoxyglucose and a Hydroalcoholic Extract from the Skin of the Fruit of *Solanum melongena* (Aubergine) Induce Selective Anticancer Activity against Melanoma CellsBy Dr. José Manuel Calderón-Montaño , Ms. Estefanía Burgos-Morón , Ms. Sara María Martínez-Sánchez , Dr. Miguel Lopez-Lazaro 

Corresponding Author

Dr. Miguel Lopez-Lazaro 

Department of Pharmacology, Faculty of Pharmacy, University of Seville, - Spain

Submitting Author

Dr. Miguel Lopez-Lazaro 

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Abstract

Prognosis of melanoma patients with metastatic disease is very poor. For example, over 95% of melanoma patients with three or more sites of metastatic disease die within 1 year. Treatment failure usually occurs because the drugs used for the treatment of these patients have a low capacity to kill melanoma cells selectively. By using human UACC-62 melanoma cells and human VH-10 skin non-malignant cells, here we show that the glycolysis inhibitors dichloroacetate and 2-deoxyglucose, and an extract from the skin of the fruit of *Solanum melongena* (aubergine, eggplant), induce selective killing of melanoma cells. This selective anticancer activity was higher than that of the anticancer drugs etoposide, 5-fluorouracil, oxaliplatin and hydroxyurea. The glycolysis inhibitor 3-bromopyruvate did not show selective cytotoxicity towards the cancer cell line. We also report that UACC-62 melanoma cells have higher glycolytic rates (glucose consumption and lactate production) than VH-10 skin non-malignant cells, which may help explain the selective cytotoxicity of the glycolysis inhibitors dichloroacetate and 2-deoxyglucose towards the melanoma cell line. Since 2-deoxyglucose and dichloroacetate have already entered clinical trials for the treatment of specific cancers, our results support their possible advancement into clinical trials for the treatment melanoma patients with metastatic disease. The selective anticancer activity of the extract from the aubergine skin warrants further investigation.

Introduction

Cancer starts as a localized disease that, when detected early, can usually be treated successfully by surgery and radiation therapy. However, the efficacy of cancer therapy decreases considerably when a primary tumor metastasizes to other parts of the body. Approximately 90% of all cancer deaths can be attributed to the metastatic spread of primary tumors, and the mean survival for people with common metastatic cancers is generally very low [1-4]. This is the case for patients diagnosed with melanoma of the skin [4-8]. It is estimated that the 5-year relative survival rates for localized melanomas is approximately 98%, while this percentage decreases to 15% when distant metastasis are observed [4]. The number of metastasis is also a prognosis factor in these patients. Indeed, data show that over 95% of melanoma patients with three or more sites of metastatic disease die within 1 year [7;8].

Despite the implementation of early detection campaigns, many melanomas are still diagnosed when cells from a primary tumor have already metastasized to other parts of the body. At this stage of disease, melanoma cells are no longer localized and can no longer be eliminated by surgery. The main form of treatment at this point is pharmacotherapy, which consist of delivering drugs systemically so that they can reach and kill the melanoma cells. Although many drugs can kill melanoma cells, most of these drugs are also toxic against non-malignant cells, cause severe side effects in patients and, therefore, need to be used at suboptimal levels. The low efficacy of cancer therapy for the treatment of patients with metastatic melanomas makes the development of selective anticancer drugs necessary.

In this paper, we have evaluated the selective cytotoxicity of several drugs by using human UACC-62 melanoma cells and human VH-10 skin non-malignant cells. We report that the glycolysis inhibitors dichloroacetate and 2-deoxyglucose, and an extract obtained from the skin of the fruit of *Solanum melongena* (aubergine, eggplant), induce selective killing of melanoma cells.

Material and Methods

Chemicals and cell lines

Dichloroacetate (98%), 2-deoxyglucose (2-deoxy-D-glucose; 98%), 3-bromopyruvate (97%), etoposide (98%), 5-fluorouracil (99%), oxaliplatin (99%) and hydroxyurea (98%) were purchased from Sigma. The human UACC-62 melanoma cell line was purchased from the American Type Culture Collection and the VH-10 skin non-malignant cell line was kindly provided by Dr. Helleday. The cell lines were maintained in DMEM supplemented with 2 mM glutamine, 50 µg/mL penicillin, 50 µg/mL streptomycin and 10% fetal bovine serum, and were cultured at 37°C in a humidified atmosphere containing 5% CO₂. Cell culture reagents were obtained from Life Technologies.

Preparation of the extracts

The exocarp (skin) of commercial fruits of *Solanum melongena* (Solanaceae) was separated from the rest of the fruit and both parts were separately extracted with ethanol:water (1:1) at 60°C for 1 hour by using an ultrasound water bath apparatus (Illustration 3 A). Ethanol was then eliminated in a rotary vacuum evaporator and the remaining water solution was lyophilized, obtaining two dry extracts.

Assay for cytotoxic activity (MTT assay)

The MTT assay is a colorimetric technique that allows the quantitative determination of cell viability. It is based on the capability of viable cells to transform the MTT salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan dye. Exponentially growing cells were seeded into 96-well plates and drugs were added 24 h later. Following an incubation period of 48 h, medium was removed and 125 µL MTT (1 mg/mL in medium) was added to each well for 4 hours. Then, 80 µL 20% SDS in 0.02 M HCl were added, plates were incubated for 10 hours at 37°C, and optical densities were measured at 540 nm on a multiwell plate spectrophotometer reader. Cell viability was expressed as percentage in relation to controls. All data were averaged from at least three independent experiments and were expressed as means ± standard error of the means (SEM).

Measurement of glycolytic rates

Glycolysis rates were assessed by measuring concentrations of glucose (initial product of glycolysis) and lactate (final product of glycolysis) in cell supernatants. Briefly, 4 x 10⁵ cells were allowed to grow in 24-well plates for 8 h. After medium removal, cells were washed with PBS and 300 µL of fresh medium were added to each well. Afterwards, cells were allowed to grow for 8 h, and glucose and lactate concentrations were determined in cell supernatants by using the Accutrend® Plus analyzer together with Accutrend glucose strips and BM-Lactate Strips (Roche Diagnostics). After calibrating the instrument with glucose and lactate calibration strips, test strips were used to determine glucose and lactate levels via colorimetric-oxidase mediator reactions according to the manufacturer's instructions [9]. Results are shown as means ± standard error of the means (SEM) of three independent experiments.

Statistical analysis

All data were averaged from at least three independent experiments and were expressed as means \pm standard error of the means (SEM). For statistical analysis we used the t-test (paired, two-tailed). A P-value >0.05 is not considered statistically significant and is not represented by any symbol. A P-value <0.05 is considered to correspond with statistical significance and is indicated with an asterisk (*), a P-value <0.01 is indicated with a double asterisk (**), and a P-value <0.001 is indicated with a triple asterisk (***).

Results and Discussion

Until the approval of ipilimumab and vemurafenib in 2011, patients with metastatic melanoma had few treatment options. Almost 50% of melanomas have mutations in oncogen BRAF (which result in constitutive activation of the MAPK pathway) and ipilimumab and vemurafenib are inhibitors of mutant BRAF [6]. Although these drugs produce substantial anti-tumor responses in patients with BRAF-mutant melanoma and prolong median overall survival to 16 months, these responses are usually not complete or durable [6]. Despite these recent advances, metastatic melanoma is still a devastating disease that requires the development of new drugs that kill melanoma cells selectively. Recent data suggest that tumor cells have metabolic alterations resulting in the constitutive activation of glycolysis despite the presence of oxygen (aerobic glycolysis or Warburg effect), and that the inhibition of glycolysis may be used to selectively kill cancer cells [10-14]. The initial aim of this work was to evaluate the selective cytotoxic activity of the glycolysis inhibitors 2-deoxyglucose, dichloroacetate and 3-bromopyruvate by using UACC-62 melanoma cells and VH-10 skin non-malignant cells. These cells were exposed for 48 h to these three glycolysis inhibitors and to the commonly used anticancer drugs oxaliplatin, 5-fluorouracil and etoposide; then cell viability was estimated with the MTT assay. Results, represented in Illustration 1, show that the glycolysis inhibitors 2-deoxyglucose and dichloroacetate induced a marked selective cytotoxicity towards the cancer cell line, which was statistically significant and higher than that induced by oxaliplatin, 5-fluorouracil and etoposide. The glycolytic inhibitor 3-bromopyruvate did not induce selective cytotoxicity towards the melanoma cell line; indeed, melanoma cells were slightly more vulnerable than non-malignant cells to the cytotoxicity of this drug (Illustration 1).

We next evaluated whether UACC-62 melanoma cells and VH-10 skin non-malignant cells had different rates of glycolysis. Illustration 2 clearly shows that the melanoma cells consumed more glucose and produced more lactate than the skin non-malignant cells. Because glucose and lactate are, respectively, the initial and final products of glycolysis, these data indicate that the melanoma cells have higher glycolytic rates than the non-malignant cells. This suggests that the melanoma cells have a higher reliance on glycolysis for their survival and, therefore, may be more vulnerable than the non-malignant skin cells to the inhibition of glycolysis induced by 2-deoxyglucose and dichloroacetate. It is not clear why the glycolysis inhibitor 3-bromopyruvate does not induce selective cytotoxicity towards the melanoma cell line. One may speculate that this drug may induce cytotoxicity against these cells through glycolysis-independent mechanisms at lower concentrations than those required to inhibit glycolysis.

Numerous reports have shown that the glycolysis inhibitors 2-deoxyglucose, dichloroacetate and 3-bromopyruvate have anticancer potential [13;15-20]. In fact, 2-deoxyglucose, and dichloroacetate have already entered clinical trials for the treatment of specific cancers (see <http://clinicaltrials.gov/>). Results shown in Illustration 1 support the possible advancement of these two glycolysis inhibitors into clinical trials for the treatment of patients with metastatic melanomas. In contrast, our data suggest that 3-bromopyruvate does not have potential for the treatment of this type of cancer.

Finally, we report that an extract from the skin of the fruit of *Solanum melongena* (aubergine, eggplant), induce selective killing of melanoma cells (Illustration 3). UACC-62 melanoma cells and VH-10 skin non-malignant cells were exposed for 48 h to an extract obtained from the aubergine skin, to an extract obtained from the rest of the fruit, and to the anticancer drug hydroxyurea; then, cell viability was estimated with the MTT assay. Results, represented in Illustration 3, show that the extract obtained from the aubergine skin induced a marked selectivity towards the cancer cell line, which was statistically significant and higher than that induced by the anticancer drugs hydroxyurea (Illustration 3) and oxaliplatin, 5-fluorouracil and etoposide (Illustration 1). The extract obtained from aubergine without skin showed certain selectivity towards the melanoma cell line; however, this activity was not statistically significant and occurred at higher concentrations (Illustration 3). Future studies are warranted to determine which constituent (or constituents) are responsible for the selective anticancer activity of the extract obtained from the aubergine skin. Previous reports suggest that delphinidin, identified in the aubergine skin [21], may play a role in this activity [22-25].

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Competing Interests

The authors declare that they do not have conflicts of interest.

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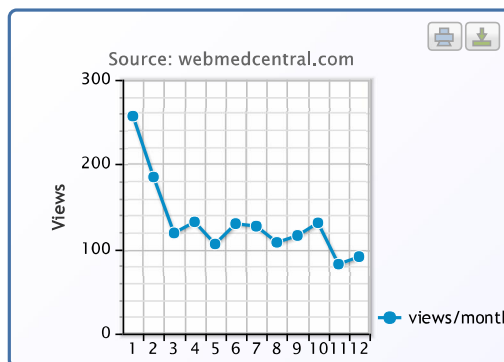
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