In Vitro and In Silico Investigations of Natural Compounds with Predicted Activity against Neuroblastomas †

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Abstract: In the present study, the ability of an ethanolic extract from Stokesia laevis to inhibit the development of human glioblastoma cell line U87 has been investigated. Cytotoxic activity has been estimated at 78% in comparison to normal human astrocyte cell line NHA, while IC50 values were 9.12 µg of gallic acid ([GAE]) equivalents per 1 mL of extract for U87 and 24.17 µg of gallic acid ([GAE]) equivalents per 1 mL of extract for NHA. Docking simulations of the active compounds in Stokesia aster and curcumin against the target anti-apoptotic protein BCL-2 has revealed the following order in the magnitude of the docking scores: native ligand > curcumin > luteolin-7-O-glucoside > luteolin-8-O-glucoside > luteolin, with root mean square (RMS) between 0.01 and 0.93. Since neuroblastomas are not restricted by the blood–brain barrier and selective cytotoxicity has been demonstrated to be higher against tumor cells than normal control astrocytes, the use of Stokesia aster ethanolic extracts and luteolin derivates as an alternative approach to neuroblastomas is to be considered.

Keywords: stokesia extract; anti-proliferative activity; glioblastoma U87; docking studies; BCL-2

1. Introduction

Glioblastoma is one of the most common and aggressive types of malignant brain tumors affecting the central nervous system in adults; current therapeutic approaches usually involve surgical intervention, chemo, and radiotherapy, but its invasive nature and high resistance to conventional therapies raise major health concerns [1]. Neuroblastoma is another form of cancer affecting nervous tissue but also some other body tissues, such as those of the lung, liver, bone marrow, adrenal gland, etc. Neuroblastomas mostly occur in children and start from immature, developing cells, namely neuroblasts, situated along the sympathetic nervous system in humans (SNS) [2]. Given the range of locations and accessibility in the human body when studying the anti-proliferative effects of nutraceuticals and active vegetal compounds [3], their bioavailability, size, and polarity must be considered; thus, in the case of glioblastoma, the size and polarity of the compounds are the key factors for their selection. In the case of neuroblastoma, the search list can be expanded with larger and more hydrophilic compounds since access to tumors is no longer restricted by the blood–brain barrier. Therefore, neuroblastomas theoretically can benefit from active compounds that normally reach the human circulatory system. In order to prove this, some recent in vitro studies using a transepithelial anti-neuroblastoma co-culture model system, in which several diluted juices from plants (kale, dandelion, lettuce, and spinach)
were applied to apical Caco-2Bbe1 cells atop dividing SH-SY5Y neuroblastoma cells, indicated inhibitory activity for kale and dandelion juices [4]. Concerning the potential active compounds, kaempferol in kale and luteolin in dandelion appear to be the most feasible anti-neuroblastoma compounds. Curcumin, a dimer of ferulic acid, was also proven to be very active against neuroblastoma tumor cell line N2a. Curcumin inhibited ERK1/2 activation by phosphorylation and, at the same time, specifically induced Bex genes to involve PI3-kinase, c-Jun N-terminal kinase JNK, and p53 associated pathways, resulting in the apoptosis of N2a neuroblastoma cells in a dose-dependent manner, with 100% cell death after a 50 µM curcumin treatment [5]. The present study has proposed to investigate in vitro and in silico the ability of an ethanolic extract from *Stokesia laevis* and corresponding active compounds, namely luteolin derivates, to inhibit the development of human glioblastoma. In vitro studies were conducted on tumor and normal brain (astrocyte) cell lines U87 and NHA, respectively, while in silico studies investigated the active compounds in stokesia against the anti-apoptotic protein BCL-2 in comparison to curcumin.

2. Materials and Methods

A medium-sized powder from the aerial part of *Stokesia laevis* was extracted with 70% (v/v) ethanol for 1 h at boiling temperature. The extractive solution was further prepared as a standardized extract (named Slae26) with a total phenolic content of 5 mg, expressed as gallic acid equivalents ([GAE]) per 1 mL of 40% (v/v) ethanol solution [6]. Slae26 was further prepared as a dilution series of 5, 10, 20, 30, 40, and 50 µg of [GAE] per 1 mL of sample. The same dilution series was prepared for the solvent sample of 40% ethanol. Both series were used for pharmacological in vitro studies.

Pharmacological in vitro studies were done on human tumor brain cell line U87 (glioblastoma) in comparison to normal human brain cell line NHA (astrocytes). Two variants of the MTS test, a cytotoxicity test and an anti-proliferative test, were carried out as described in the technical bulletin from the Promega Corporation on the CellTiter 96 AQueous One Solution Cell Proliferation Assay [7]. At approx. 70% (cytotoxicity test) and 30% (anti-proliferative test) confluence of the cells’ cultures (U87 and NHA), Slae26 and 40% ethanol dilution series were applied and allowed to act for 24 and 48 h. The culture medium was then removed, and the cells were incubated with MTS solution for another 2 h. Finally, the absorbance at 490 nm of the test sample (Slae26 series) in comparison with the control solvent sample (40% ethanol series) of each cell culture studied (U87 and NHA) was measured using BMR-100 Microplate Reader (Boeco, Germany). The recorded values were used for both cell viability and cell anti-proliferative activity estimation. The results can be presented as % of cell viability (see formula below) or simply optical density (O.D.) at 490 nm along with the total phenolic content in the samples (µg/mL).

\[
\% \text{cell viability} = \frac{A_{490 \text{ of treated cells}}}{A_{490 \text{ of control cells}}} \times 100
\]

Docking simulations have been performed using CLC Drug, Discovery Workbench (QIAGEN, Aarhus, Denmark) software on the anti-apoptotic protein BCL-2 in complex with an acyl-sulfonamide-based ligand, PDB ID 2O2F [8]. The simulation protocol was previously validated [9]. The intermolecular interactions that occurred mainly reflect the hydrogen bonding of the investigated ligands (curcumin, luteolin, luteolin-7-O-glucoside, luteolin-8-C-glucoside) in complex with the BCL-2 fragment in the binding pocket of 36.35 Å³. Results are given as docking scores as functions of the distance of the identified hydrogen bonds and the strength of the potentially formed complexes.

3. Results and Discussions

Figure 1 presents the results of the cytotoxicity study at 24 h for the Slae26 dilution series and the solvent sample (40% ethanol) dilution series tested on human tumor brain cell line U87 in comparison to normal human brain cell line NHA. The cytotoxicity diagram at 24 h indicates that the influence of the solvent sample (40% ethanol) upon U87 tumor...
cells (blue line) and NHA normal cells (red line) is similar and of low intensity. However, the Slae26 test sample induced a decrease in the viability of both types of brain cells in culture, although more augmented in human tumor brain cell line U87 (gray line) than normal brain cell line NHA (yellow line). Table 1 shows the cytotoxicity study results computed as a percentage of inhibitory activity against the two brain cell lines along with the total phenolic concentration in the Slae26 dilution series. The dynamic of the inhibitory activity suggests Slae26 selective activity against brain cells at concentrations between 10 and 30 μg of [GAE]/mL and the possibility of decreasing the dilution of the samples towards 5 μg/mL.

Table 1. Cytotoxic activity of Slae26 against NHA normal and U87 tumor brain cells.

<table>
<thead>
<tr>
<th>Cytotoxicity Study/Cell Line</th>
<th>Inhibitory Activity (%) of Slae26 against the Dilution Series (10–50 μg of [GAE]/mL of Sample)</th>
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<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>NHA</td>
<td>23.27</td>
</tr>
<tr>
<td>U87</td>
<td>39.86</td>
</tr>
</tbody>
</table>

Figure 2 presents the results of the anti-proliferative study at 24 h and 48 h for the Slae26 dilution series and the solvent sample (40% ethanol) dilution series tested on human tumor brain cell line U87. As opposed to the cytotoxicity study, the anti-proliferative study has underlined the capacity of the ethanol solvent on its own to induce the decrease in the viability of tumor brain cell line U87 in culture. There were registered independent activities at 24 h (blue line) and 48 h (red line), with a medium of inhibitory activity against the dilution series at about 44%. The Slae26 dilution series also induced an augmented decreases in tumor brain cell U87 viability in culture at 24 h (gray line) and 48 h (red line) after treatment. The average values for anti-proliferative, inhibitory activity against U87 tumor brain cells were computed at 60.62% in the first 24 h and 35.28% in the next 24 h, with a maximum of 78% inhibitory activity.
Table 2 shows the results as the percentages of inhibition at 24 and 48 h along with the total phenolic content in the Slae26 dilution series.

<table>
<thead>
<tr>
<th>Anti-Proliferative Study at 24 h and 8 h, Resp.</th>
<th>Inhibitory Activity (%) of Slae26 against the Dilution Series (µg of [GAE]/mL of Sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>U87–24 h</td>
<td>18.44</td>
</tr>
<tr>
<td>U87–48 h</td>
<td>37.73</td>
</tr>
</tbody>
</table>

Figure 2 reveals the resulting docking scores for the studied compounds as a measure of the stability of their complexes with the BCL-2 protein, and Figure 4 illustrates the hydrogen bond interactions formed by curcumin, showing the structure revealing the greatest binding affinity and exhibiting a docking score of −63.88. Interactions occurred with PHE109 and VAL130 amino acid residues on the A chain.
Figure 4. Hydrogen binding of curcumin within the binding pocket.

4. Conclusions

The active compounds in *Stokesia laevis* are luteolin, luteolin-7-O-glucoside, and luteolin-8-C-glucoside. Judging by the results of pharmacological in vitro studies on the Slae26 standardized extract dilution series from 5 to 50 μg of [GAE]/mL of sample, the resulting IC50 values were 9.12 μg of [GAE]/mL of extract for human tumor brain cell line U87 and 24.17 μg of [GAE]/mL of extract for human normal brain cell line NHA, and anti-proliferative activity against tumor cell line U87 reached 78%. The opportunity to consider *Stokesia laevis* extracts and the main active compounds in ethanolic extracts (luteolin derivate) in further across-the-board applications for glioblastoma has been revealed. The use of *Stokesia laevis* extracts and the main active compounds from ethanolic extracts, such as luteolin derivate, on glioblastoma cell lines shows promise for future applications. The results of the docking approach can be further supplemented with studies on other, more specific targets. Moreover, considering the previous studies [5] on *Stokesia laevis* ethanolic extracts (e.g., on human colon cancer cell line Caco-2/IC50 = 36 μg of GAE/mL of extract, human tumor breast cell line BT20/IC50 = 42 μg of GAE/mL of extract, and murine melanoma cell line B16/IC50 = 39 μg of GAE/mL of extract), which showed positive outcomes, the extension to the vivo level would be an insightful approach.

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