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Novel synthetic chalcones induces apoptosis in human glioblastoma cells

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ABSTRACT

Glioblastoma multiforme is the main and most frequent tumor in adults' central nervous system. With a survival average of 5% two years after diagnosis, this type of cancer is a main health problem. Substances like the chalcones have been tested in order to develop new treatments. Here, we studied the effects of three synthetic chalcones (A23, C31 and J11) on A172 and surgery obtained-glioma cells. All chalcones showed a decrease in cell viability, mainly C31. An increase in apoptosis levels with no further increase of necrosis was observed. This augmentation may be linked to the high oxidative effect found, caused by the increased presence of reactive oxygen species and nitric oxide production. Cell cycle distribution showed an arrest at G0/G1 and S phases, suggesting that C31 interferes in cell cycle control. Our results shall aid in directing future research with this substance and its antitumor effect.

1. Introduction

Malignant gliomas are tumors, which arise from astrocyte lineage cells, and they are considered the most common and devastating primary brain tumors, representing 50%–60% of all central nervous system (CNS) neoplasms [31]. As a result of high proliferation, invasiveness and resistance to the radiation displayed by the severest form, the glioblastoma multiforme (GBM) prognosis is low and the mean survival time is less than 12 months. Globally, the incidence of this type of cancer is variable. The country with the highest rate of incidence for 100,000 people is Finland with 6.3%, and the one with the lowest rate is Japan, where 1.8% are affected. In the American continent, the incidence is 5.2% in the USA and 4.2% in Brazil, both considered very high incidences by the World Health Organization [10,29]. Despite the intense efforts to develop treatments, effective options are not yet available [23].

The induction of selective apoptosis is the primary objective of radio and chemotherapy, and the resistance observed in gliomas can be caused by a failure in the apoptosis initiation pathways. Many homeostatic balances, in various tissues, are mediated by

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apoptosis as well as proliferation and differentiation. An unbalance in these processes may lead to the formation of tumors by excessive cell division, poor cell cycle control, mutations on checkpoints, and many others [43]. Therefore, treatment strategies along with new anti-neoplastic drugs, which have greater specificity in inducing apoptosis in tumor cells, are needed not only to improve the cure rate, but also patients' life quality [14].

Chalcones are precursors of the flavonoid biosynthesis in plants, and can be prepared synthetically by condensing aryl ketones with aromatic aldehydes in the presence of condensing agents [26]. These molecules have been used in the synthesis of a variety of synthetic heterocyclic compounds [5], and have showed several biological effects, which suggest potential pharmaceutical uses as antimicrobial [24], anti-inflammatory [7], analgesic [40], antileukemic and antitumoral agents [6,19,36]. Several studies also shown that chalcones have anti-proliferative and cytotoxic effects, and may arrest the cell cycle in different tumor cell lines, blocking cell cycle in G2/M phase [30,34], or in G0/G1 phase, leading to an apoptotic cell death [16,33].

In previous studies, a series of synthetic chalcones derived from quinoxalines have shown a strong cytotoxic effect in human U-138 MG glioma cells, with IC_{50} (Inhibitory Concentration of 50%) values ranging from 2.29 to 2.67 μg/ml [22]. Also, another study screened the inhibitory activity of geranyl prenylated chalcone on C6, U87 MB, CNS-1 and 13-06 MB glioma cell lines. Using cell cycle distribution, DNA fragmentation, chromatin condensation and protein expression as apoptosis indicators, significant results were observed with an IC_{50} of 20 μg/ml of chalcones in all four cell lines. The proliferation aspect was also accessed and showed significant reduction [39]. Other studies have also shown that cell death induced by chalcones are caused by heavy induction of autophagy, in lung cancer [45], as well as autophagy-mediated necroptosis (RIP1- and RIP3-dependent necrosis) [15] and in association with the chemotherapeutic cisplatin, caused the Ripoptosome-mediated apoptosis activation [38].

The main purpose of this study was to assess the cytotoxic effect of three completely new and unpublished synthetic chalcones A23 [(2*E*)-1-(3′, 4′, 5′-trimethoxy-phenyl)-3-(1-naphthyl)-2-propen-1-one], C31 [(2*E*)-1-(3′-methoxy, 4′-hydroxy-phenyl)-3-(2-naphthyl)-2-propen-1-one] and J11 [(2*E*)-1-(2′, 4′, 5′-trimethoxy-phenyl)-3-(1-naphthyl)-2-propen-1-one] on a glioblastoma cell culture (GBM1) obtained from human sample after surgical resection. This cell culture was evaluated by cytogenetic and showed typical cancer hallmarks like the loss of heterozygosis on chromosome 1p and 10q, and many other secondary rearrangements [17]. The other cell culture used as well as the commercial human glioma cell line A172. C31 is more promising than the other chalcones already tested, since it showed a higher cell viability decrease than A23 and J11, enhancing the apoptosis levels without increasing necrosis. These effects can be explained, at least partially, by alterations in cell cycle and increased ROS and NO levels.

2. Materials and methods

2.1. Primary glioma culture

The sample was obtained from a resection surgery of *glioblastoma multiforme* on a patient at the Celso Ramos Hospital, in Florianópolis, Santa Catarina, Brazil. The obtained sample was collected in 15 ml conical tube containing Dullbeco's Modified Eagle's medium and nutrient mixture F12 (DMEM-F12; Invitrogen), with 10% fetal bovine serum (FBS; Cultilab). Then, in a laminar flow cabinet, the blood vessels and cerebral membranes were removed. The sample was tripsinyzed (Tripsin/EDTA, 0.05%; Gibco) and homogenized to be plated in 25 cm^2 culture flasks, containing DMEM F-12 with 10% FBS, at 37 °C in culture conditions (atmosphere of 5% CO_2/95% air). To assure the cell culture did not contain unspecific cell types, we started the experiments after at least 10 passages. When the 80% of confluence was reached, the cells were tripsinyzed and plated in 96 wells plates (10^4 cells/well) for experimentations. *All the procedures were approved by the local ethics committee for human research (CEP – 108.286).*

2.2. A172 cell line culture

The glioma cell line A172 was cultured in DMEM F-12, supplemented with 10% FBS in 25 cm^2 culture flasks, at 37 °C in culture conditions [35]. When 80% of confluence was reached, the cells were tripsinyzed and plated in 96 wells plates (10^4 cells/well) for experimentations.

2.3. Murine astrocyte culture

The primary cultures of astrocytes were prepared from cerebral cortex obtained from newborn (0–3-day-old) Wistar rats as previously described [21]. Briefly, the astrocytes were plated into 96-well plates (3.5×10^5 cells) and grown to 80% confluence (10–14 days) in the presence of DMEM-F12, and supplemented with 10% FBS. The cell cultures were incubated at 37 °C in a humidified 5% CO_2 and 95% atmosphere. *All the procedures were approved by the local ethics committee for animal research (CEUA – PP00582).*

2.4. Chalcone synthesis

The chalcones A23, C31 and J11 (Fig. 1) were prepared by an aldol condensation between corresponding aldehydes (1 mmol) and acetophenones (1 mmol) using methanol as solvent in basic conditions (KOH 50% p/v). The reaction was maintained under magnetic stirring at room temperature for 24 h, and when finished, distilled water and HCl 10% were added to acidify and facilitate the precipitation of the compounds. After vacuum filtration, the reaction products were purified by recrystallization in dichloromethane and hexane. Structures were identified by melting points, infrared spectroscopy, hydrogen and carbon magnetic resonance and elemental analysis. The chemical characterization of A23, C31 and J11 was previously published [8] [4].

2.5. Cell treatment

Cells were treated in two different ways, depending on the experiment. A172, GBM1 and astrocyte cells were treated with the chalcones A23, C31 and J11, in the following concentrations: 1, 10, 25, 50, 75 or 100 μM, for 24, 48 or 72 h, for the cell viability curves. Vehicle-controls were assayed with the same volume of DMSO (dimethyl sulfoxide, Vetec, max of 0.4%). In another set of experiments, A172 and GBM1 cells were treated with the IC_{50} obtained from the viability curves' data, for 24 h prior to the experiments.

2.6. Cell viability assay

The cell viability was determined through the MTT (3-(4,5-Diamethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay [27]. After a 24-h treatment, the medium was removed and the cells were incubated with a solution of MTT (0.2 mg/ml in PBS; Sigma) for 2 h, at 37 °C. Then, the MTT solution was removed, the formazan crystals were dissolved in DMSO, and the viability was quantified spectrophotometrically at a wavelength of 540 nm, with a multimode reader Infinite M200 TECAN.