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Research paper

Curcumin-loaded lipid-core nanocapsules as a strategy to improve pharmacological efficacy of curcumin in glioma treatment

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ABSTRACT

In this study, we developed curcumin-loaded lipid-core nanocapsules (C-LNCs) in an attempt to improve the antiglioma activity of this polyphenol. C-LNC showed nanotechnological properties such as nanometric mean size (196 nm), 100% encapsulation efficiency, polydispersity index below 0.1, and negative zeta potential. The *in vitro* release assays demonstrated a controlled release of curcumin from lipid-core nanocapsules. In C6 and U251MG gliomas, C-LNC promoted a biphasic delivery of curcumin: the first peak occurred early in the treatment (1–3 h), whereas the onset of the second phase occurred after 48 h. In C6 cells, the cytotoxicity of C-LNC was comparable to non-encapsulated curcumin only after 96 h, whereas C-LNCs were more cytotoxic than non-encapsulated curcumin after 24 h of incubation in U251MG. Induction of G2/M arrest and autophagy were observed in C-LNC as well as in free-curcumin treatments. In rats bearing C6 gliomas, C-LNC (1.5 mg/kg/day, i.p.) decreased the tumor size and malignance and prolonged animal survival when compared to same dose of non-encapsulated drug. In addition, serum markers of tissue toxicity and histological parameters were not altered. Considered overall, the data suggest that the nanoencapsulation of curcumin in LNC is an important strategy to improve its pharmacological efficacy in the treatment of gliomas.

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1. Introduction

Gliomas are the most common, aggressive, and difficult type of primary brain tumor to treat. Despite recent advances in chemotherapeutic strategies to treat other types of cancer, gliomas frequently resist contemporary drugs, and prognosis remains dismal [1,2]. A number of dysregulated cell signaling cascades have been described in gliomas, including the Ras/MEK/ERK, JAK/STAT3, NFkappaB, and PI3K/Akt pathways. Dysregulation of these pathways is driven by mutation, amplification, and overexpression of multiple genes such as PTEN, EGFR, PDGFR-a, p53, and mTOR [2–4]. In addition, the privileged location of these tumors limits the efficacy of classical chemotherapeutics due to restrictions imposed by the blood brain barrier (BBB) [1–3]. These characteristics together contribute to the high rates of growth and invasiveness observed in gliomas culminating in median survival of ~14 months after diagnosis [1–4]. Thus, the development of new therapeutic strategies to treat this disease is urgently required.

For decades, curcumin has been considered one of the most promising natural compounds for the treatment of diverse types of maladies including cancer and neurodegenerative diseases [5]. Previous studies by our group [6,7] and others [8–14] have demonstrated that curcumin can inhibit the growth of several glioma cell lines through a variety of mechanisms involved in cell cycle progression (induction of p21 and p53, inhibition of cyclin D1), invasiveness (blocking of secretion of MMPs), anti/proapoptotic response (decreases in bcl-2, bcl-xL, and caspases activation), autophagy and modulation of survival pathways such as JAK/STAT3, EGFR, PI3K/Akt, and NFkappaB [7–14]. Previous studies reported by our group [6,7] and by Dhandapani et al. [14] showed

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that curcumin is selectively cytotoxic to tumor cells, but it spares normal neural cells such as astrocytes and neurons.

Despite promising *in vitro* tests, the potential of curcumin to limit glioma growth *in vivo* remains poorly described [7–9,13]. Summarizing the *in vivo* findings, we recently reported that a 10-day treatment with 50 mg/kg/day of curcumin decreased brain-implanted C6 tumors in Wistar rats [7]. Similar results were obtained in U87-implanted mice treated with 100 mg/kg/day curcumin [13] and in mice bearing tumors derived from implanted glioma-initiating cells treated with 300 mg/kg curcumin [8]. Others reported the ability of dietary curcumin (0.05%/w ad libitum) to inhibit the growth of Tu-9648-brain implants in C6B3F1 mice [9]. In human clinical trials, high doses of oral curcumin brought clear benefits in familial adenomatous polyposis (FAP), whereas its potential in colorectal, pancreatic, breast, and other cancers remains open or inconclusive due to the reduced number of patients enrolled in the studies [15–19]. In this regard, there is a consensus that the *in vivo* potential of curcumin probably remains underestimated and the development of new formulations could be useful to improve its therapeutic potential [20,21].

Much of the knowledge regarding the actions of curcumin was obtained from *in vitro* models of cell growth in variable drug concentrations, which are almost impossible to reach under *in vivo* conditions [7–13]. Particularly in cases of cancer, despite the promising results in cultured cancer cell lines at concentrations as low as 10 μM, curcumin blood levels do not reach these concentrations via dietary consumption in humans due to its poor aqueous solubility, limited mucosal absorption and first-pass metabolism, evidencing a gap between basic findings and clinical applications of this drug [22]. Results from experiments in the Caco-2 cell line have classified curcumin as a Biopharmaceutics Classification System (BCS) class IV molecule; this information allows the prediction of the rate-limiting step involved in intestinal absorption after oral administration of curcumin [23]. In addition, the susceptibility of curcumin to degrade at alkaline and neutral pH and in the presence of light has been demonstrated [24].

Several authors have designed drug delivery systems, such as solid lipid nanoparticles [25,26], phospholipid and cyclodextrin complexes [27,28] and polymeric nanoparticles, aimed at enhancing the bioavailability and therapeutic potential of curcumin [5,29]. Among the numerous benefits that nanoparticles possess, it is important to highlight the demonstrated ability of these carriers in increasing the delivery of drugs to the brain, this being a promising strategy for the treatment of diseases affecting the central nervous system [20,21,30–32]. The exact mechanism whereby nanoparticles enhance the transport of drugs across the brain–blood barrier (BBB) is unclear. Previous studies have reported that the polysorbate 80 coating is a suitable strategy for brain delivery aimed at improving the receptor-mediated endocytosis of nanoparticles at the level of the BBB [21,32]. Recently, two important studies showed an enhanced transport, increased retention, and sustained release of curcumin to the brain when this polyphenol was associated with polymeric nanoparticles [20,21].

In the present study, we tested a new type of particle developed by our group called lipid-core nanocapsules (LNCs). These particles are able to improve the stability of formulations and exert photoprotective effects [33–37]. In previous studies, LNCs have increased the biodistribution of trans-resveratrol in different tissues, including the brain [34], and promoted a significant reduction in the growth of malignant gliomas by increasing intracerebral levels of the nonesteroidal anti-inflammatory indomethacin [32]. In an attempt to improve the antiglioma potential of curcumin, we formulated curcumin-loaded lipid-core nanocapsules (C-LNCs) and evaluated their physicochemical characteristics, uptake and cytotoxicity to C6 and U251MG glioma cells *in vitro* and, mainly, the potential to inhibit the growth of brain-implanted gliomas *in vivo*. The effects of C-LNC and non-encapsulated curcumin were compared.

2. Materials and methods

2.1. Reagents

Curcumin, poly(ε-caprolactone) (PCL), and sorbitan monoestearate were obtained from Sigma–Aldrich (São Paulo, Brazil); grape seed oil was acquired from Delaware (Porto Alegre, Brazil); polysorbate 80 was purchased from Vetec (Rio de Janeiro, Brazil) and acetone from Nuclear (São Paulo, Brazil). HPLC grade acetonitrile was obtained from Tedia (São Paulo, Brazil). All chemicals and solvents were of pharmaceutical or HPLC grade and used as received. Propidium iodide, EDTA, acridine orange, trypsin, and MTT (3-(4,5-dimethyl)-2,5-diphenyl tetrazolium bromide) were supplied by Sigma–Aldrich (São Paulo, Brazil), and reagents for cell cultures were purchased from Difco (Detroit, MI, USA).

2.2. Preparation of lipid-core nanocapsules

The lipid-core nanocapsules were prepared by interfacial deposition of preformed polymer [36,37]. PCL (0.1 g), grape seed oil (165 μL), sorbitan monostearate (0.0385 g), and curcumin (0.005 g) were dissolved at 40 °C in 27 mL of acetone. The organic phase was injected, under stirring, into the aqueous phase consisting of polysorbate 80 (0.077 g) and water (54 mL). The suspension was kept under stirring for 10 min, and then, the acetone was evaporated and the final volume (10 mL) of the suspension was adjusted using reduced pressure. This formulation was called C-LNC. Blank lipid-core nanocapsules (B-LNCs) were prepared in the same way, omitting the presence of the drug. The formulations were prepared in triplicate and stored at room temperature and protected from light (amber glass flasks).

2.3. Characterization of lipid-core nanocapsules

2.3.1. Particle size analysis, polydispersity index, and zeta potential

Particle size and the polydispersity index were determined at 25 °C by photon correlation spectroscopy (Zetasizer Nano ZS®, Malvern Instruments, Malvern, UK) after previous dilution of the nanoparticles with ultrapure water. The zeta potential values were estimated on the basis of electrophoretic mobility on the same equipment at 25 °C, after dilution of the samples in a 10 mM NaCl aqueous solution. To ensure that the particle size of the formulations was submicrometric, the suspensions were also analyzed by laser diffraction (Mastersizer® 2000, Malvern Instruments, Malvern, UK). The results were expressed by the number size distribution (%) of the particles, considering the recent definition of the European Commission for nanomaterials [38].

2.3.2. Nanoparticle tracking analysis (NTA)

For visualization of the C-LNC, nanoparticle tracking analysis (NTA) experiments were carried out (NanoSight LM 10 & NTA 2.2 Analytical Software, Nanosight Ltd., Amesbury, UK). After appropriate dilution of the C-LNC in ultrapure water, the sample was introduced into the Nanosight sample chamber with a disposable syringe. The samples were measured at room temperature for 60 s with automatic detection. When the particles suspended in fluid are irradiated by a laser source, they scatter light and the image can be captured by a charge-coupled device (CCD) camera. The software is able to identify and track each individual particle, and it calculates the particle diameter from its Brownian motion [39,40].

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