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New celecoxib multiparticulate systems to improve glioblastoma treatment

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ABSTRACT

Treatment of malignant gliomas consists of resection followed by radiotherapy and chemotherapy. Celecoxib (CXB), a selective COX-2 inhibitor, is able to control inflammation and pain, to improve the efficacy of radiotherapy, and to inhibit at high doses the growth of cancer cells. Two new delivery systems for CXB are developed: microspheres (MPs) for implantation in the brain after partial/complete removal of the tumor, and nanoparticles (NPs) for their potential to cross the blood brain barrier and deliver CXB into the CNS. Cell culture assays performed in PC12, SKN-AS and U373-MG cells demonstrate the antiproliferative affects of CXB, with EC50 values of 99.81 μM and 82.4 μM in U373-MG and SKN-AS cells. Encapsulation efficacy of CXB in formulation MP2 (20% CXB) was 74.6 ± 2.2% with a zero-order release rate of 47.8 μg/day/20 mg microspheres for 34 days. Uncoated and polysorbate 80-coated CXB-NPs are prepared by nanoprecipitation. Mean sizes of uncoated and coated CXB-NPs were 173.6 ± 44.9 nm and 100.6 ± 62.1 nm. Cerebral cortex images showed a marked increase of fluorescence when the surfactant-coated NPs were administered to rats. These results suggest that both CXB formulations (MPs and NPs) are adequate systems to enhance the effects of chemotherapy in the treatment of malignant brain tumor.

1. Introduction

Malignant brain tumors are usually secondary cancers that grow very fast and spread to other areas of the brain and spine. Regarding glioma, it is the most common type of malignant primary brain tumor, representing more than half of all primary brain tumors.

Glioblastoma multiforme (GBM) refers to a heterogeneous group of neoplasms with different location in the CNS. It is fast growing and can occur at any age, but mainly affects adults between 45 and 70 years old (Morshed et al., 2013). GBM is responsible for around 50% of all primary gliomas in adults being the second cause of cancer death in adults (<35 years old) (Allard et al., 2009). The survival and growth of GBM are related to an adequate blood supply with malignant gliomas being highly vascularized.

The treatment for GBM depends on the size and type of the tumor as well as on its rate of growth and state of health of the patient. The typical treatment of malignant gliomas consists of resection which causes inflammation (Stupp and Roila, 2009). The presence of edema results in increased pressure in the brain, which can cause more symptoms than the tumor itself. After surgery the options of treatment include radiotherapy and chemotherapy. The former clearly results in patient survival but it can induce inflammatory responses that may be indistinguishable from a relapse of the disease probably due to a transient increase in the permeability of the tumor vasculature.

Steroids can be utilized to reduce and improve neurological symptoms with these agents being administered at the lowest dose in order to avoid long-term adverse effects such as hyperglycemia, myopathy, iatrogenic cushing, osteoporosis and capillary fragility. These symptoms may be especially severe in the case of patients who require rehabilitation to regain mobility after surgery (Wen and Kesari, 2008).

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NSAIDs have been used for a long time to reduce fever, inflammation and pain. Recent studies have shown that many cyclooxygenase (COX) inhibitors can act as potential agents for the prevention and treatment of cancer. The group of selective COX-2 inhibitors has the advantage of acting on specific molecular targets. A high level of COX-2 expression is associated with more aggressive types of tumors such as GBM, being a strong predictor of poor survival. Among NSAIDs celecoxib (CXB) is a selective COX-2 inhibitor which in addition to effectively modulate inflammation and pain, has proven to be efficient at high doses as an inhibitor of the growth of cancer cells (Harris et al., 2000; Sadeghi-Aliabadi et al., 2013). For instance, Nam et al. (Nam et al., 2004), using an orthotopic rat gliosarcoma model found that orally given CXB significantly reduced the incidence and size of tumors. They also found that tumor cells obtained from rats treated with CXB exhibited lower levels of phospho-Akt/PKB, an alternative target of this drug. In this regard there are several studies and clinical trials devoted to clarify the role that CXB could play in reducing certain tumors when used in combination with chemotherapy (Peulen et al., 2012; Zhang et al., 2013).

CXB is also being evaluated as a radiosensitizer agent especially taking into account some reports that indicate that its radiosensitizing effects take place in tumor cells with elevated levels of COX-2 (Shin et al., 2005). These effects have also been observed in experimental tumors in which COX-2 expression was restricted to the tumor neovasculature in which CXB was effective in reducing cancer progression when combined with radiotherapy (Ma et al., 2011).

However, systemic administration of CXB results in numerous adverse effects, particularly in patients with preexisting renal disease and heart failure. Furthermore the severity of these effects augments with the high doses that are needed to achieve the anti-inflammatory and radiosensitizing effects of the drug.

Encapsulation of active ingredients allows for the design of controlled release systems developed to achieve and maintain therapeutic drug concentrations over a long period of time using a single dose, in order to eliminate or minimize the maximum concentrations that exceed the therapeutic requirements and increase adverse effects (Varde and Park, 2004; Park et al., 2005; Fernández et al., 2011; Garbayo et al., 2011).

For this we have developed and characterized two new controlled release systems of CXB: microparticles (MPs) and nanoparticles (NPs). CXB-MPs are developed for their implantation in the brain after partial or complete removal of the tumor, thereby reducing inflammation and/or post-traumatic edema, and cellular proliferation and improving the efficacy of radiotherapy. Since the blood-brain barrier (BBB) plays an important role in limiting strategies of therapy, we have developed CXB-NPs which after being able to cross the BBB will reduce both inflammation and cellular proliferation and to enhance the effects of radiation. Furthermore, in our work the antiproliferative activity of CXB is evaluated in various cell lines.

2. Materials and methods

2.1. Materials

Celecoxib (CXB) was supplied by Hangzhou Onion Chemical Co. Ltd. (China), PLGA 50:50 poly(D,L-lactide-co-glycolide) (Resomer® RG 502, M_w = 12 kDa (GPC)) and PLGA 50:50 poly(D,L-lactide-co-glycolide) (Resomer® RG 503, M_w = 72 kDa (GPC)) were obtained from Evonik (Germany). Polyvinyl alcohol (PVA) (M_w = 49 kDa) was obtained from Merck (Germany) and polysorbate 80 from Panreac (Spain). All other reagents were of analytical grade and provided by Merck (Germany). Distilled and deionized water Milli-Q (Millipore Corporation, USA) was used in the preparation of all buffers and solutions.

2.2. Preparation of microspheres

Microspheres (MPs) were prepared by the solvent evaporation technique from an O/W emulsion using 200 mg of PLGA Resomer® 503. For this, the corresponding amount of PLGA was dissolved in 1 mL CH_2Cl_2 by vortex mixing. A fixed amount of CXB (20 or 40 mg) was dissolved in the organic phase under agitation to obtain a drug:polymer ratio of 10% (formulation MP1) and 20% (formulation MP2), respectively (Table 1). The external phase of the emulsion consisted of 1% aqueous solution of PVA (10 mL). The organic phase was emulsified in the external aqueous phase using a homogenizer (Polytron PT 3000 Kinematica®, USA) at 3000 rpm for 3 min. The immature MPs were suspended in 10 mL of 0.1% aqueous solution of PVA and the system was continuously stirred for 3 h at room temperature to allow complete evaporation of the organic solvent.

Finally, MPs were vacuum filtered using 5 μm filters Millipore® SMWP (Millipore, USA), washed with distilled water (50 mL) and freeze-dried for 3 h (Flexi-Dry™; SP Industries, Inc., USA). Freeze drying conditions were 30 °C and 100 mTorr.

Blank MPs were also obtained (formulation MP3). All formulations were prepared in triplicate.

2.3. Preparation of nanoparticles

CXB-loaded PLGA nanoparticles (formulation NP1) were prepared by a nanoprecipitation method using an acetone–water system. Briefly, an amount of CXB (5 mg) and 50 mg of PLGA RG® 502 were dissolved in 4 mL of acetone by vortexing (2 min). This mixture was added dropwise into 12 mL of 0.5% PVA under stirring (15 min). The resulting suspension was then evaporated with rotavapor (Buchi Coop, Switzerland) to completely remove acetone (30 min, 25 °C and 70 mbar). The nanoparticle suspension obtained is washed and centrifuged at 15,000 rpm for 30 min (Avanti J-301, Beckman Coulter Inc., USA), and NPs were washed three times to remove PVA. Finally, the dispersed solution was freeze-dried for 24 h (Flexi-Dry MP™, FTS® Systems, USA). Blank PLGA nanoparticles (formulation NP2) and 5% rhodamine PLGA nanoparticles (formulation NP3) were also prepared using the same procedure.

Moreover, CXB-loaded PLGA-polysorbate 80 nanoparticles (formulation NP4). were prepared by the same nanoprecipitation method used for elaboration of formulation NP1 but incorporating 1% polysorbate 80 into 12 mL of 0.5% PVA. Blank PLGA-polysorbate 80 nanoparticles (formulation NP5) and 5% rhodamine PLGA-polysorbate 80 nanoparticles (formulation NP6) were also prepared using the same procedure.

2.4. Characterization of MPs and NPs

2.4.1. Morphology of microspheres and nanoparticles

Shape and surface morphology of MPs and NPs were analyzed by scanning electron microscopy (JEOL JEM 6335F, Jeol Ltd., Japan).

Table 1
Formulations developed.

Formulation	Description
MP1	10% CXB-loaded PLGA microparticles
MP2	20% CXB-loaded PLGA microparticles
MP3	Blank PLGA microparticles
NP1	CXB-loaded PLGA nanoparticles
NP2	Blank PLGA nanoparticles
NP3	Rhodamine PLGA nanoparticles
NP4	CXB-loaded PLGA-polysorbate 80 nanoparticles
NP5	Blank PLGA-polysorbate 80 nanoparticles
NP6	Rhodamine PLGA-polysorbate 80 nanoparticles