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Original article

Induction of cancer cell death by apoptosis and slow release of 5-fluoracil from metal-organic frameworks Cu-BTC

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ABSTRACT

This study aimed to evaluate the mechanism associated with cytotoxic activity displayed by the drug 5-fluorouracil incorporated in Cu-BTC MOF and its slow delivery from the Cu-BTC MOF. Structural characterization encompasses elemental analysis (CHNS), differential scanning calorimetry (DSC), thermogravimetric analysis (TG/DTG), Fournier transform infrared (FIT-IR) and X-ray diffraction (XRD) was performed to verify the process of association between the drug 5-FU and Cu-BTC MOF. Flow cytometry was done to indicate that apoptosis is the mechanism responsible for the cell death. The release profile of the drug 5-FU from Cu-BTC MOF for 48 hours was obeisant. Also, the anti-inflammatory activity was evaluated by the peritonitis testing and the production of nitric oxide and pro-inflammatory cytokines were measured. The chemical characterization of the material indicated the presence of drug associated with the coordination network in a proportion of 0.82 g 5-FU per 1.0 g of Cu-BTC MOF. The cytotoxic tests were carried out against four cell lines: NCI-H292, MCF-7, HT29 and HL60. The Cu-BTC MOF associated drug was extremely cytotoxic against the human breast cancer adenocarcinoma (MCF-7) cell line and against human acute promyelocytic leukemia cells (HL60), cancer cells were killed by apoptosis mechanisms. The drug demonstrated a slow release profile where 82% of the drug was released in 48 hours. The results indicated that the drug incorporated in Cu-BTC MOF decreased significantly the number of leukocytes in the peritoneal cavity of rodents as well as reduced levels of cytokines and nitric oxide production.

1. Introduction

5-fluorouracil (5-FU), a pyrimidine analog, is one of the broad spectrum anticancer drugs [1–3] used in the treatment of malignancies like glioblastoma [4] and breast cancer [5]. Since 5-FU interferes with DNA synthesis, it principally acts as a thymidylate synthase inhibitor [6,7]. However, short half-life, wide distribution, and various side effects limit its medical applicability [8–10]. To overcome the above-mentioned limitations, an ample number of studies has been carried out on sustained drug delivery systems for 5-fluorouracil.

Rahman et al. prepared and evaluate the colon-specific microspheres of 5-fluorouracil for the treatment of colon cancer. The authors observed that the results clearly indicate that there is great potential in delivery of 5-FU to the colonic region as an alternative to the conventional dosage form [11].

Lekha-Nair et al. studied the biological evaluation of 5-fluorouracil nanoparticles for cancer chemotherapy and its dependence on the PLGA carrier (nanoparticles with dependence on the lactide/glycolide combination of PLGA). 5-FU-entrapped PLGA nanoparticles showed smaller size with a high encapsulation efficiency [12]. Horcajada et al. reported the efficiency of some MOFs as carriers of drugs was tested using antiviral and antitumor drugs: bulsufan, azidothymidine-trisfofato, cidovir and doxorubicin. According to the authors, incorporation of some antitumor drugs used for cancers such as leukemia and myeloma into the MIL-100 MOF was considered extremely high. [13].

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Extensive research within the last two decades has revealed that most chronic illnesses, including cancer, diabetes, and cardiovascular and pulmonary diseases, are mediated through chronic inflammation. Thus, suppressing chronic inflammation has the potential to delay, prevent, and even treat various chronic diseases, including cancer, several drugs including: dexamethasone and doxorubicin have been packaged as nanoparticles and proven to be useful in "nano-chemoprevention" and "nano-chemotherapy" [13,14].

Thus in this perspective, this study aimed to evaluate the action mechanism associated with the antitumor activity of the 5-fluorouracil incorporated into Cu-BTC and a possible anti-inflammatory activity, since studies in the literature show a close relationship between inflammation and the appearance of new cancers.

2. Materials and methods

2.1. System drug-MOF used

The association was performed using 5-FU 99% (Sigma Aldrich) and the Cu-BTC MOF – BasoliteTM C300 (688814) produced by BASF. First the drug (300 mg) was dissolved in 150 mL of Milli-Q ultrapure water and 100 mg of Cu-BTC were added, in a proportion of 3:1 (w/w – drug/MOF). The resulting suspension was kept under stirring at room temperature for seven days. Aliquots were removed after seven days, then centrifuged (4500 rpm) for 20 minutes and the supernatant was analysed by UV-Vis spectroscopy to determine the amount of drug present in the metal organic framework. The analysis was performed in triplicate.

2.2. Chemical characterization

Elemental analysis (CHNS) measurements were performed in a CE Instruments elemental analyser, model EA1110. FIT-IR spectra were collected from KBr discs utilizing a Bruker spectrophotometer (IFS-66) with Fourier transform (spectral range 4000 to 400 cm^{-1}). The results were analyzed using the OPUS Spectroscopic Software from Bruker. TG/DTG curves were obtained in the temperature range between 20–900 °C using a Shimadzu TGA 50 thermobalance under a dynamic nitrogen atmosphere (50 mL min^{-1}) at a heating rate of 10 °C min^{-1} and an alumina crucible containing approximately 3 mg of the sample. The instrument calibration was performed before the tests using a calcium oxalate monohydrate standard, according to the American Society for Testing and Materials. The DSC data were recorded in a DSC 50 cell (Shimadzu) in the 25–600 °C temperature range under a dynamic nitrogen atmosphere (100 mL min^{-1}) in an alumina crucible containing 2.0 mg of the sample; a heating rate of 10 °C min^{-1} was used. The powder patter XDR were obtained in a Bruker D8 Advance X-Ray diffract meter (Kα(Cu) 1,54 Å), in the range 5° to 50°, step 0.02° and acquisition time 1 second.

2.3. Delivery study (in vitro)

For in vitro release study was used transparent hard gelatin capsules containing 110.97 mg of incorporation, which was equivalent to 50 mg of 5-FU. Dissolution (dissolutor, Varian, model VK-7000/7010/750D) was performed in PBS (pH: 6.8), volume of 500 mL at 37° ± 0.5 and screw speed of 100 RPM. The collections were performed on days 0.5, 1.5, 5.5, 15, 20, 24, 39, 44 and 48 hours. At every given time, a sample was collected from 2.5 mL to be analyzed on HPLC, followed by replacement of the dissolution medium. The samples were filtered on Millipore® membrane 0.22 mm in pore size, 13 mm (Millex). All analyzes were performed in triplicate.

The samples obtained from dissolution studies were individually subjected to analysis by HPLC – DAD using isocratic mobile phase 85% of acetonitrile: 15% of water (v/v) with a 2 mL/min flow, the oven temperature was 25 °C, with a stationary phase C18 column (250 × 4.6 mm/5 μm), and 20 μL injection volume of the samples. The LC system used was a high performance liquid chromatograph (HPLC) Shimadzu® equipped with a quaternary pump model LC – 20ADVP, powered by helium degasser model DGU – 20A, PDA detector model SPD – 20AVP, oven model CTO-20A SVP, auto sampler model SIL – 20A DVP and controller model SCL – 20AVP. The data were processed by software Shimadzu® LC solution 2.0.

2.4. Cytotoxic activity and apoptosis tests verification

The cytotoxic tests were carried out against four cell lines: NCI-H292 cells (lung mucoepidermoid carcinoma), MCF-7 cells (breast adenocarcinoma), HT29 cells (colon adenocarcinoma) and HL60 cells (promyelocytic leukemia). The cells were maintained in DMEM – Dulbecco's Modified Eagle Medium. Samples were considered cytotoxic when the rates of cell growth inhibition exceeded 40%, according to the protocol established by Geran [15].

The apoptosis test was done used 5 μg/mL and 10 μg/mL. HL60 (3 × 105 cells/mL) were plated onto 24-well tissue culture plates and treated with (Cu-BTC MOF; 5-fluorouracil (5-FU) and 5-FU + Cu-BTC). After the 72 h incubation period, cells were pelleted and resuspended in 1× Binding Buffer. The pellet was incubated for 10 minutes with 5 μL of the annexin-V and 10 μL of the propidium iodide (Annexin V-FITC Apoptosis Detection Kit-SIGMA).

Cell fluorescence was determined by flow cytometry in a FACS Calibur cytometer (Becton, Dickinson and Company, New Jersey, USA) using the Cell Quest software. Ten thousand events were evaluated per experiment and cellular debris was omitted from the analysis.

2.5. Animals used in the pharmacological tests

For the tests, Swiss albino female mice (*Mus musculus*) were used, weighing between 30–35 g, with an average age of two months. The animals were monitored according to the norms of the National Institute of Health Guide for Care and Use of Laboratory Animals. The experiments were conducted according to the National Cancer Institute protocol [16] and approved by the UFPE-Animal Experimentation Ethic Committee: 23076.024149/2012-48.

2.6. Peritonitis induced by carrageenan

In this assay, were used six groups of six animals, treated groups received orally 25, 50 and 75 mg/kg of (5-FU + Cu-BTC MOF). The others group received orally saline vehicle (0.1 mL/10 g) used as negative control and dexamethasone (0.5 mg/kg) as positive control. One hour after the treatment, the inflammation was induced by intraperitoneal (i.p) application of 0.1 mL/10 g of carrageenan (1% in saline solution).

After 4 hours the animals were euthanized in a chamber of CO_2 so was injected into the peritoneal cavity 3 mL of phosphate buffered saline PBS containing EDTA to collect the peritoneal fluids. The total leukocyte number was determined in hematology analyzer Micros 60®. The exudates were centrifuged and the supernatant stored at −20 °C for analysis of nitric oxide and citokines levels [17].

2.7. Analysis of nitric oxide production

The nitrite accumulated in the samples was measured as an indicator of NO production using the Griess reaction. Briefly, the