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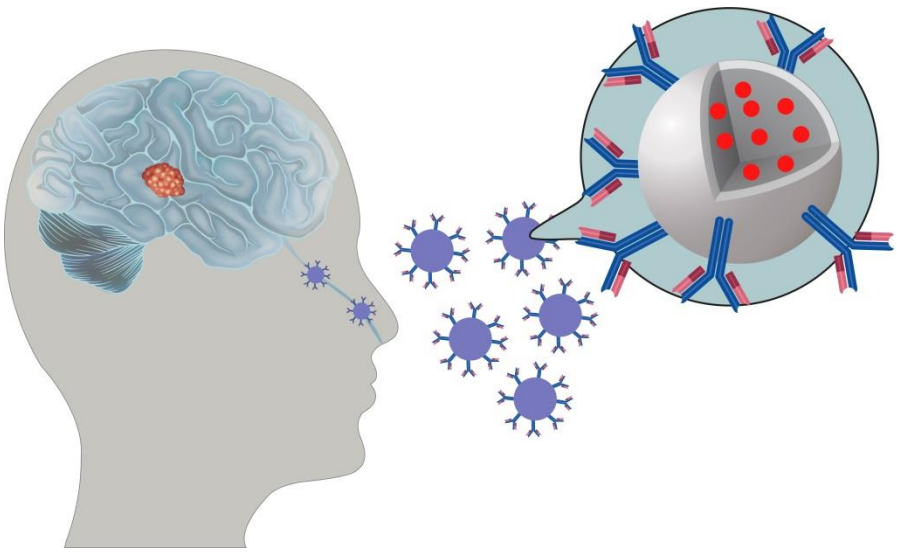
Desenvolvimento de sistemas multifuncionais nanoestruturados para a liberação de fármacos administrados por via nasal no tratamento de glioblastoma



Natália Noronha Ferreira Naddeo

Orientadora: Profª. Drª. Maria Palmira Daflon Gremião

Coorientadora: Profª. Drª. Maria de Fátima Monginho Baltazar

Araraquara-SP

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Nanostructured multifunctional systems for drug release through the nasal route applied on glioblastoma treatment

Natália Noronha Ferreira Naddeo

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TÍTULO DA TESE: DESENVOLVIMENTO DE SISTEMAS MULTIFUNCIONAIS NANOESTRUTURADOS PARA A LIBERAÇÃO DE FÁRMACOS ADMINISTRADOS POR VIA NASAL NO TRATAMENTO DE GLIOBLASTOMA

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Araraquara, 30 de março de 2020

DEDICATION

This study is wholeheartedly dedicated to my beloved parents, who have been my source of inspiration and gave me strength when I thought of giving up, giving me also moral, spiritual, emotional and financial support;

I also dedicate all efforts and achievements reported here to my lovely husband and daughter, Bruno and Helena, whose are, and will always be, my inspiration.

Mommy loves you.

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“Nothing in life is to be feared,
it is only to be understood.
Now is the time to understand more,
so that we may fear less”.

MARIE CURIE

RESUMO

Glioblastomas (GBM) representam 77% dos tumores malignos do sistema nervoso central (SNC) e ainda hoje, apesar de todos os avanços na terapia, continua com prognóstico limitado. A existência de barreiras fisiológicas como a barreira hematoencefálica (BHE) representa o principal obstáculo que impede que concentrações adequadas do fármaco atinjam o local de ação. Por suas vantagens anatômicas, uma estratégia proposta para a administração de fármacos destinados ao SNC consiste no uso da via nasal. Além disso, o uso de terapias combinadas utilizando fármacos capazes de agir em diferentes alvos moleculares deve ser considerada para o tratamento de doenças complexas como GBM. O candidato a fármaco ácido alfa-ciano-4-hidroxicinâmico (CHC) e o anticorpo monoclonal cetuximab (CTX) já são explorados devido à capacidade de agir em diferentes alvos moleculares nas células tumorais e aplicados em conjunto, como uma nova abordagem combinada, podem melhorar os resultados terapêuticos. De forma complementar, a utilização de sistemas de liberação baseados em nanotecnologia trará inevitavelmente ganhos terapêuticos à combinação proposta, permitindo que atributos específicos sejam agregados ao sistema e possibilite não somente a administração nasal, como também a associação de diferentes fármacos em um único carreador. Assim, o presente estudo propõe o desenvolvimento de diferentes plataformas poliméricas baseadas em poli(ácido láctico-co-glicólico) (PLGA) e quitosana trimetilada (TMC) ou quitosana oligomérica (OCS) para encapsulação do CHC. Ambos os sistemas desenvolvidos contendo CHC encapsulado exibiram tamanho de aproximadamente 300 a 400 nm contendo quitosana em sua superfície mais externa (potencial zeta positivo) e uma alta porcentagem de CHC encapsulado (± 85%). A caracterização físico-química dos sistemas mostrou sua estabilidade coloidal, principalmente na presença do fármaco CHC. Dados de DRX sugerem que a interação entre CHC e as nanopartículas (NPs) de PLGA/OCS apresentam padrões diferentes das NPs de PLGA/TMC. A conjugação entre CTX e NPs foi realizada através de ligações supramoleculares e covalente resultando em 85 e 58% de eficiência, respectivamente. A análise da eficácia terapêutica utilizando protocolos *in vitro* empregando linhas celulares de glioma U251 e SW1088, estabeleceu que, comparando ambos os sistemas conjugados, o PLGA/OCS parece ter uma capacidade terapêutica mais relevante. Por esse motivo, esse sistema foi selecionado para as futuras investigações. Análise de imunoblot confirmou que o CTX associado às NPs continua a exercer sua eficácia terapêutica. A análise da atividade antiangiogênica, desenvolvimento e progressão do tumor usando modelo de membrana corioalantóica de embrião de galinha (CAM) revelaram uma tendência de redução do tumor quando NPs conjugadas foram utilizadas. Além disso, elas também exibiram uma atividade antiangiogênica. O perfil de liberação *in vitro* mostrou que a liberação de CHC foi sustentada e

retardada pelo seu encapsulamento nas NPs. Estudos de permeação *ex vivo* utilizando mucosa nasal suína mostraram que a permeação de CHC foi modificada pela sua inclusão nos sistemas. A avaliação da capacidade de NPs administradas pela via nasal em atingir o SNC utilizando tomografia de fluorescência forneceu evidências de independentemente do procedimento de conjugação, NPs foram eficazes em realizar o transporte direto para o cérebro pela rota nasal. Portanto, considerando todos os resultados mencionados, o sistema desenvolvido exibiu um conjunto de atributos favoráveis que as tornam alternativas promissoras a serem futuramente consideradas no tratamento de GBM.

PALAVRAS CHAVE: glioblastoma; administração nasal; ácido α-ciano-4-hidroxicinâmico; cetuximabe; nova estratégia terapêutica; efeito combinatório; nanotecnologia.

ABSTRACT

Glioblastomas (GBM) account for 77% of malignant tumors in the central nervous system (SNC), and today, despite all advances in therapy, remains with a limited prognosis. The existence of physiological barriers as the blood brain barrier (BBB) represents the main obstacle that limits appropriate concentrations of drugs designed to therapy. Due to their anatomical advantages, a strategy proposed for direct delivery to SNC involves the use of the nose-to-brain route. Besides, combination therapy that uses multiple drugs against different molecular targets should be considered for complex diseases such as GBM. Drugs like alpha-cyano-4-hydroxycinnamic acid (CHC) and the monoclonal antibody cetuximab (CTX) are already explored for their capacity to act against different hallmarks of cancer and applied together, as a novel combining approach, might improve therapeutic outcomes. Therefore, advances in nanotechnology-based delivery systems will inevitably bring therapeutic gains to the proposed combination since they enable acquisition of important characteristics desired and also the association of different drugs into a single carrier. Thus, the current study proposes the development of different polymeric platforms based on poly(lactic-co-glycolic acid) (PLGA) and trimethyl chitosan (TMC) /chitosan oligosaccharide (OCS) for CHC encapsulation. Both CHC-loaded developed systems (PLGA/TMC and PLGA/OCS) exhibited nanostructure organization of about 300 to 400 nm, containing chitosan on their outermost surface (positive zeta potential) and a high percentage of CHC encapsulation (±85%). Physicochemical characteristics have shown great colloidal stability, especially in the presence of CHC drug. DRX data suggest that interaction between CHC and PLGA/OCS NPs follows patterns different than PLGA/TMC NPs. Conjugation between CTX and developed CHC-loaded NPs was optimally obtained by supramolecular forces and covalent bonds, resulting in 85 and 58% of efficacy, respectively. Analysis of therapeutic efficacy using *in vitro* protocols employing U251 and SW1088 glioma cell lines, established that, comparing both conjugated systems, PLGA/OCS seems to have greatly therapeutic capacity. Therefore, this system was chosen to further investigations. Blot analysis confirmed that CTX associated with NPs continues to exert its therapeutic efficacy. Analysis of antiangiogenic activity, tumor development, and progression using the chicken chorioallantoic membrane disclosed a trend of tumor reduction when conjugated NPs were employed. In addition, this system also exhibited antiangiogenic activity. *In vitro* release profile showed that CHC release was sustained and retarded by drug encapsulation into NPs. The *ex vivo* permeation study applying nasal porcine mucosal showed that CHC permeation was delayed by the inclusion of system complexity. Analysis of NPs delivery using fluorescence tomography provides evidence that the developed PLGA/OCS NPs, independently of conjugation procedure,

were effective in providing nose-to-brain transport. Taking into consideration all of the aforementioned results, we anticipate that the developed system exhibited a set of favorable attributes that make them promising alternatives to be further considered in GBM treatment.

KEY WORKS: glioblastoma; nose-to-brain delivery; α-cyano-4-hydroxycinnamic acid; cetuximab; novel therapeutic strategy; combinatory effect; nanotechnology.

LIST OF ABBREVIATION

ANOVA- Analysis of variance

ANVISA- Brazilian National Agency for Sanitary Surveillance

BBB - Blood brain barrier

CAM - Chicken Chorioallantoic Membrane

CED - Convection-enhanced delivery

CHC - α-cyano-4-hydroxycinnamic acid

CNS - Central nervous system

CS - Chitosan

CTX – Cetuximab

DDS - drug delivery systems

DFT - Density functional theory

DLS: Dynamic light scattering

DMEM - Dulbecco's Modified Eagle's Medium

DMF- N,N-Dimethylformamide

DMSO - dimethyl sulphoxide

EE% - Entrapment efficiency

EGFR - Epidermal growth factor receptor

EMA - European Medicines Agency

ERK - Extracellular signal-regulated kinase

FBS - Fetal bovine serum

FDA - Food and Drug Administration

FEG-SEM - Field emission scanning electron microscopy

FTIR - Fourier transform infrared

GBM – Glioblastoma

HIF-1α - Hypoxia-inducible factor-1 alpha

HNSCC - Head and neck squamous cell carcinoma

HPLC - High performance liquid chromatography

IC_{50} - Half maximal inhibitory concentration

IR-ATR - Attenuated total reflectance infrared spectroscopy

LDHA - Lactate dehydrogenase A

MAbs - Monoclonal antibodies

Mal-PEG-NHS - N-Hydroxysuccinimide-PEGmaleimide

MCTs - Monocarboxylate transporters

MTIC - 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide

NPs - Nanoparticles

OCS - Chitosan oligosaccharide

P-AKT – Phosphorylated protein kinase B

PARP- poly (ADP-ribose) polymerase

PDI - polydispersity index

P-EGFR – Phosphorylated epidermal grow factor receptor

P-ERK1/2 - Phosphorylated extracellular signal-regulated kinases

Pen-Strep - Penicillin/streptomycin

PKM2 - Pyruvate kinase M2

PLGA – Poly (d,l-lactide-*co*glycolide

RSD - Relative standard deviation

RTK - Tyrosine kinase receptors

SATA - N-succinimidyl S-acetylthioacetate

SD – Standard deviation

SRB - Sulforhodamine B

TBS-T - Tris-Buffered Saline/0.1% Tween 20

TCA- trichloroacetic acid

TEM - Transmission electron microscopy

TFA - Trifluoroacetic acid

TMC - N,N,N-trimethyl chitosan

TMZ - Temozolomide

WHO -World Health Organization

XRD - X-ray diffraction

ZP- Zeta potential

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SUMMARY

1. INTRODUÇÃO

Entre os tumores cerebrais, o glioblastoma (GBM) representa a forma mais maligna e letal, sendo uma das principais causas de morte relacionadas ao câncer (Colen *et al.*, 2011). Extremamente agressivo altamente invasivo e neurologicamente destrutivo, em sua manifestação, a sobrevida média é de 12 a 16 meses, dados estatísticos que permanecem inalterados nos últimos anos, apesar da descoberta de novos fármacos e avanços tecnológicos (Maher *et al.*, 2001; Karsy, Michael *et al.*, 2018).

Alguns fármacos tem demonstrado potencial atividade no tratamento de GBM. Entretanto, poucos agentes quimioterápicos possuem aprovação pela Food and Drug Adminitration (FDA). Embora novas vias moleculares sejam publicadas todos os dias, aprimorando nosso conhecimento sobre a capacidade biológica e invasiva das células de GBM, a transposição da ciência básica para prática clínica não segue a mesma tendência (Veliz et al., 2015). Tratamentos eficazes que visam atingir o sistema nervoso central (SNC) ainda representam um grande desafio para a comunidade científica, levando em consideração a incapacidade de muitos fármacos em acessar o cérebro (Begley, 2004). A presença de barreiras fisiológicas, principalmente a barreira hematoencefálica (BHE), limita acessibilidade do fármaco, dificultando a eficácia de terapias medicamentosas (Portnow et al., 2009; Alexander et al., 2019). Assim, muitos candidatos a fármacos que provaram ser eficazes em seus locais de ação, falharam e foram descartados durante os ensaios clínicos (Begley, 2004).

Até o momento, o protocolo de tratamento padrão para GBM se inicia com intervenção cirúrgica seguida de radioterapia combinada com a administração oral de temozolomida (TMZ) que provou ser um dos agentes antineoplásicos mais eficazes (Lee; Ooi , 2016). No entanto, embora esse agente alquilante possua a capacidade de atravessar o BHE, sua meia-vida curta requer altas doses que devem ser administradas pela via oral para garantir níveis terapêuticos no cérebro, o que, por sua vez, traz vários efeitos colaterais (Portnow et al. 2009). Apesar disso, poucas mudanças e melhorias na sobrevida foram relatadas (Gately et al., 2018).

A ativação anormal do receptor do fator de crescimento epidérmico (EGFR) ocorre em aproximadamente 40% dos casos de GBM (Roskoski, 2014; Hicks *et al.*, 2016). Assim muitos ensaios clínicos em andamento exploram a utilização de fármacos direcionados para este alvo (Roskoski, 2014). O Cetuximabe (CTX) é um anticorpo monoclonal IgG1 quimérico que liga-se ao segmento extracelular do domínio III ligado ao EGFR, inibindo a ativação de várias vias de transdução de sinal que estão associadas ao desenvolvimento tumoral, progressão, disseminação

metastática e diminuição da sobrevida. Além disso, o CTX também regula a expressão do fator de crescimento endothelial vascular (VEGF), o que pode dificultar o processo de angiogênese (Roskoski, 2014).

Adicionalmente, uma característica importante das células tumorais consiste na ocorrência de uma reprogramação no seu metabolismo energético. A intensa proliferação celular requer um ajuste capaz de fornecer a energia necessária ao crescimento e divisão celular. Como este suprimento energético deve ser rapidamente produzido, as células tumorais substituem a respiração aeróbica pela glicólise anaeróbica, mesmo na presença de oxigênio, um fenômeno registrado pela primeira vez por Otto Warburg (Warburg, 1930; Warburg, 1956; Hanahan; Weinberg, 2011). Como consequência do designado "efeito Warburg", as células cancerígenas secretam lactato / H^+ (ácido lático) que deve ser exportado para o ambiente externo para manutenção do pH intracelular (Gillies *et al.*, 2008; Hanahan; Weinberg, 2011; Miranda-Goncalves *et al.*, 2013; Amorim *et al.*, 2015). Os gliomas malignos são altamente glicolíticos, produzindo altos níveis de ácido lático que devem ser externalizados para o meio extracelular através de transportadores específicos evitando a morte das células por acidose.

As proteínas transportadoras de monocarboxilato (MCTs) compreendem uma família de 14 membros em que 4 isoformas estão relacionadas ao transporte transmembrana de prótons (Izumi *et al.*, 2003). A inibição da atividade dos MCTs, que interrompe o estado de homeostasia por alterações de pH, vem sendo apontada como um dos novos alvos a ser explorado no tratamento de tumores cerebrais, como GBM. O ácido α-ciano-4-hidroxicinâmico (CHC), um monocarboxilato aromático de 189,2 $g.mol^{-1}$ promove uma inibição competitiva da atividade de MCTs em mamíferos sem apresentar citotoxicidade aparente *in vivo* (Colen *et al.*, 2006; Colen *et al.*, 2011).

Estudos anteriores demonstraram que o uso de CHC contra diferentes células de glioma diminuiu o metabolismo glicolítico, a migração e a invasão, induzindo a morte celular (Miranda-Goncalves et al., 2013). A avaliação do CHC como estratégia terapêutica em tumores cerebrais usando entrega por convecção (CED) demonstrou inibição *in vivo* do crescimento do tumor e a ocorrência de necrose completa do tecido tumoral em modelo animal (Colen et al., 2011).

Embora esses resultados sejam bastante encorajadores, a molécula CHC apresenta uma solubilidade bastante limitada o que deve comprometer seu efeito terapêutico quando administrada por vias de administração convencionais como a oral. Na tentativa de superar essas limitações, o CHC já foi incorporado em estruturas de zeólitos, sólidos cristalinos com estruturas microporosas muito regulares nas quais substâncias químicas ativas podem ser incluídos, para o desenvolvimento de sistemas de liberação aplicados ao tratamento do câncer. Como resultado, o encapsulamento de

CHC nestes sistemas também forneceu um aumento significativo de sua capacidade terapêutica (Vilaça *et al.*, 2011). Por outro lado, a administração sistêmica de CTX reduz a proliferação celular em uma série de células tumorais (Van Den Eynde *et al.*, 2011) e, embora essa realidade possa ser estendida ao GBM, a terapia anti-EGFR usando CTX é limitada pela incapacidade deste fármaco em atingir o sítio de ação em concentrações adequadas para que se tenha sua eficácia terapêutica (Eller *et al.*, 2005; Hicks *et al.*, 2016).

A expansão do conhecimento e a compreensão sobre as características moleculares do GBM demonstra um comportamento altamente agressivo e adaptativo, sugerindo que um único agente terapêutico não trará avanços significativos na sua terapia. Assim, a abordagem de múltiplos alvos através de fármacos capazes de agir em diferentes vias de sinalização, de maneira simultânea, pode fornecer um cenário mais favorável no tratamento. Uma série de protocolos de terapia utilizando fármacos associados já foi testada, no entanto, uma associação destinada a inibir o EGFR juntamente com MCTs ainda não foi estudada para GBM.

Muitas vezes, compreender um efeito terapêutico, unicamente, não determina o êxito de uma terapia, especialmente para GBM. Diferentes ensaios clínicos empregando novas substâncias terapêuticas únicas não têm mostrado resultados encorajadores. O principal fator associado à falha terapêutica está associado à BHE, que torna o cérebro praticamente inacessível a grandes moléculas polares (Benoit et al., 2000; Lalatsa; Barbu, 2016). Por outro lado, a via nasal de administração oferece uma alternativa não invasiva interessante para uma conexão direta com o cérebro. Devido a suas características tais como fácil administração, rápida absorção e automedicação, essa nova abordagem está se intensificando (Romeo et al., 1998; Alex et al., 2015).

A administração de fármacos pela via nasal para atingir o cérebro é possível pela localização anatômica da cavidade nasal que contem uma região onde o neuroepitélio está exposto ao ambiente externo possibilitando o rápido transporte do fármaco para o cérebro (Haque *et al.*, 2014). Entretanto, é necessário pontuar que esta via de administração apresenta alguns desafios como a permeabilidade deste epitélio e o tempo de residência da formulação na cavidade nasal. Assim, esforços consideráveis têm sido explorados para o desenvolvimento de sistemas projetados para administração nasal (Alex *et al.*, 2015).

Na área da tecnologia farmacêutica, a proposta de reformulação de um antigo fármaco como uma nova estratégia terapêutica e atividade biológica aprimorada tem sido o foco principal de muitos pesquisadores. Como os resultados desejados a uma terapia não se baseiam apenas no perfil farmacocinético e farmacodinâmico do fármaco, mas também em sua biodisponibilidade no sítio de ação, nas respostas biológicas e nos resultados terapêuticos esperados relacionados à abordagem da

entrega, especialmente para doenças complexas como GBM. Plataformas tecnológicas baseadas em polímeros permitem o desenvolvimento de sistemas inovadores com uma ampla gama de propriedades físico-químicas e estruturais, que podem ser delineadas para atingir características específicas e desejadas para a administração de fármacos. A compartimentalização do fármaco em um ambiente restrito também é uma estratégia promissora para modular propriedades biofarmacêuticas em que atributos críticos, como proteção contra degradação e interação biológica, podem ser projetados de acordo com as necessidades específicas. Para esta finalidade, uma variedade de biomateriais vem sendo proposta. Entre eles, polímeros naturais e sintéticos têm atraído cada vez mais atenção devido à sua grande variedade de estruturas. Sua versatilidade também permite a associação de diferentes fármacos produzindo um carreador único com propriedades desejáveis à via de administração pretendida.

Nano ou microestruturas baseadas em PLGA e quitosana provaram melhorar significativamente a interação do fármaco com as células tumorais, promovendo o aumento de sua citotoxicidade (Chakravarthi; Robinson, 2011). Além disso, estas plataformas já foram destacadas por proporcionarem o transporte de diferentes fármacos ao cérebro através da via nasal uma vez que podem modular a interação desses sistemas com a mucosa interferindo na permeabilidade de diferentes fármacos (Tong et al., 2017; Meng et al., 2018).

Levando em consideração todos os desafios apresentados acima para o tratamento com GBM e tentando estimular o desenvolvimento de novas estratégias terapêuticas que possam melhorar desempenho terapêutico, primeiro levantamos a hipótese de que dois fármacos já estudados (CHC e CTX) poderiam fornecer efeitos aditivos ou sinérgicos contra o GBM. Adicionalmente, os sistemas nanoestruturados para a liberação de fármacos foram introduzidos para fornecer ganhos terapêuticos uma vez que permitem a modulação de propriedades específicas estruturais e biofarmacêuticas para melhorar a resposta biológica desejada. Além disso, devido a sua versatilidade, carreadores baseados em polímeros podem proporcionar a associação de diferentes substancias em um único sistema.

Assim o presente estudo foi desenvolvido para investigar a possibilidade de uma nova terapia combinada para GBM aliada à nanotecnologia por meio da entrega direta ao cérebro através da via nasal de administração. Nanopartículas (NPs) de PLGA e quitosana foram inicialmente desenvolvidas para o encapsulamento do fármaco CHC. Em seguida, uma completa caracterização físico-química foi realizada com o objetivo de comparar as duas plataformas. Posteriormente, CTX foi conjugado na superfície dessas nanoestruturas utilizando diferentes estratégias. A prova de conceito foi investigada pela análise do desempenho terapêutico *in vitro* utilizando cultura de

células nas linhas celulares de glioma SW1088 e U251. Para finalizar, a resposta biológica e a entrega do sistema através da via nasal foram investigadas utilizando protocolos *in vivo.*

of positively charged molecules provide stronger adherence to the mucus layer. Interestingly, the potential benefit of this adhesive/retention behavior remains unclear. Nevertheless, both surface charges are proven to provide the transport (Bonaccorso *et al.*, 2017).

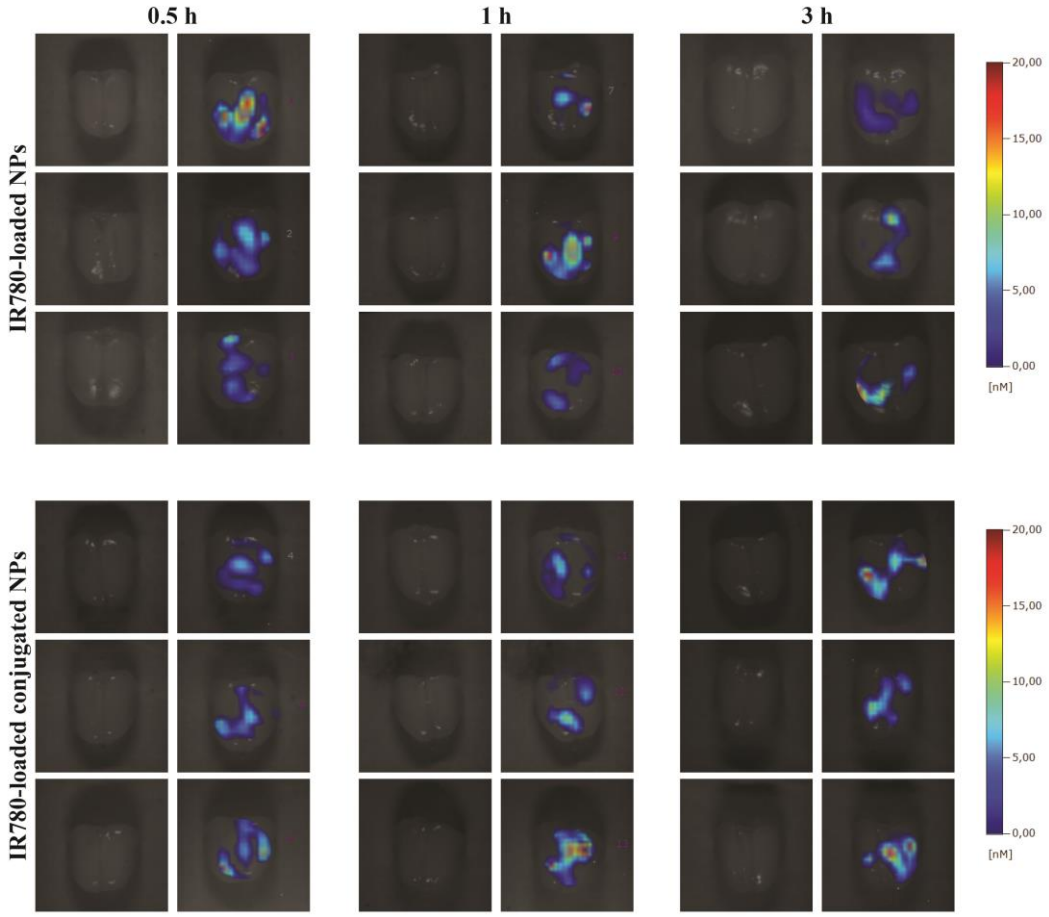

Figure 30: *Ex vivo* brain fluorescence tomography. Pictures were taken 0.5, 1 and 3 hours after intranasal administration of IR780-loaded NPs and IR780-loaded conjugated NPs; (n=3). First column represents the negative controls.

Taken together, these data provides evidence that developed nanoparticles of PLGA/OCS in the range of 250 to 310 nm of size, were effective to provide nose-to-brain transport, independent of the conjugated procedure. Futures studies are needed to quantify the dimension of transport provided by these systems.

7. CONCLUSIONS

The present study has provided the first investigation of a dual therapeutic strategy combining CHC and CTX against GBM, using the SW1088 and U251 glioma cell lines. Besides, nanotechnology has brought important features to the proposed strategy, considering that

nanostructured systems are able to optimize the therapeutic effect, especially for complex pathological processes.

In this work, we have successfully rationally developed innovative delivery systems based on PLGA and chitosan (PLGA/TMC and PLGA/OCS) polymeric nanoparticles for CHC encapsulation. Both developed platforms resulted in a nanostructured organization at about 300 to 400 nm of size containing chitosan on its outermost surface, what led to a positive ZP, and a high percentage of CHC encapsulation.

Complete physicochemical characterizations have shown that CHC drug could modify the kinetic aspects of NPs stabilization. In addition, interaction between CHC drug and PLGA/OCS NPs follows different patterns than PLGA/TMC NPs. Therefore, conjugation between CTX and developed NPs was optimally obtained by supramolecular forces (PLGA/TMC NPs) at pH 6.5 and covalent bonds (PLGA/OCS NPs), resulting in 85 and 58% of efficacy, respectively.

Comparing both developed systems in terms of cell viability and metabolism assay, PLGA/OCS NPs showed more promising results than those obtained with PLGA/TMC NPs. For that reason, this system was chosen to proceed the scientific investigation.

In vitro release profile showed that CHC release was modified by the drug encapsulation into NPs. Additionally, both release profile correlated better with the Weibull model following a complex release mechanism. The *ex vivo* permeation study using nasal porcine mucosa has shown that CHC permeation was delayed by the inclusion of system complexity.

Blot analysis confirmed that CTX associated to PLGA/OCS NPs by covalent bonds were biologically active in addition to reinforce that system efficacy was specific and not associated to toxicity. Analysis of antiangiogenic activity, tumor development and progression using the CAM assay disclose a trend for tumor reduction when conjugated NPs were employed as therapeutic strategy, in addition to provide a significative reduction in the blood vessel number compared with all other applied treatment.

Analysis of nose-to-brain delivery using fluorescence tomography provided evidence that developed NPs, regardless of the CTX conjugation procedure, were effectively transported from nose to the brain.

Taking into consideration all of the aforementioned results, we anticipate that the developed CHC-loaded PLGA/OCS NPs conjugated with CTX by covalent bonds, exhibited a set of favorable attributes that make them promising alternatives to be further explored and considered in GBM treatment.

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