Enhancing contrast agents and radiotracers performance through hyaluronic acid-coating in neuroradiology and nuclear medicine

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Abstract
The use of hyaluronic acid nanoshells has been proposed to encapsulate prodrugs and exploit the mechanisms of interactions between living cells, like endocytes or cancer cells and hyaluronic acid, which is a natural component of the extracellular matrix. In this review we describe the potential and the limits of this promising research trend and discuss the theoretical advantages of such an engineering approach. Is it a possible scalability to increase the efficacy and biodegradability of molecules like contrast media and radiotracers especially for neuroradiology and nuclear medicine studies.

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Introduction

One of the rapidly growing research fields referring to conventional and high-Tesla magnetic resonance imaging (MRI) and nuclear medicine studies is the study of new contrast agents and radiotracers capable to early detect microaggregates of cancer cells and cancer recurrence. Creation and development of solid tumors, of circulating tumor cells (CTC) and of early metastases may thus be a reality in the future

Interaction between hyaluronic acid nanoparticles and living cells

Since hyaluronic acid (HA) has immunoneutrality, others [2, 4] and we [3, 5], proposed it as a biocompatible and biodegradable material for tissue engineering and for the development of delivery of various drug systems. Recently, formulations of several drugs or prodrugs conjugated to polymeric coated HA nanoparticles of poly(ε-coprolactone), polylactide, poly(lactic-co-glycolic acid), polyethylene-glycol, polycarylates and chitosan were found effective as smart delivery systems both in vitro and in vivo [5-9].

Hyaluronic acid is a natural linear polysaccharide constituted by repeating units of N-acetyl-D-glucosamine and D-glucuronic acid with monosaccharides and linked together by alternating β-1,3 and β-1,4 glycosidic bonds. The carboxyl groups of HA are predominantly ionized at pH 7.4 and therefore in physiological conditions, HA appears as a polyanion, known as hyaluronan [10]. Hyaluronic acid is found in a wide range of molecular weights ranging from 20kDa of the HA oligomers (o-HA), to the high-molecular weight (HMW) of bulk HA (~1.5MDa). In solution, the chains of HA adopt a random coil conformation, and its high hydrophilic nature leads to multiple hydrogen bonds with H_2O, explaining the viscous and elastic characteristics of the connective tissues in which this polysaccharide is abundant. Besides chemical conjugation, it has been proved that HA can also be linked to other prodrugs or to proper delivery systems by weak interactions such as those involved in the formation of ion pairs [11, 12] expanding the number of possible candidates for conjugation with or encapsulation within those nanocarriers.

Hyaluronic acid conjugates could leverage on their propensity to overcome the blood brain barrier (BBB), so that first to produce biologic effects on the central nervous system (CNS) and secondly to provide a specific tumor targeting activity, which takes advantage of the peculiar interaction between HA receptors on the bilipidic membrane of glioma cells and the enzymes for HA degradation contained in the extracellular matrix (ECM). The first, is mainly based on receptor-mediated endocytosis of HA nanoparticles at the level of brain capillary endothelial cells [13]. The second, represents the basis for

the matrix metalloproteinases (MMP)-triggered release of contrast agents in proximity to cancer cells aggregates [14, 15]. So, HA can be useful in neuroradiology and neuroncology.(Figure 1. Artistic representation of a CD44 transmembrane receptor).

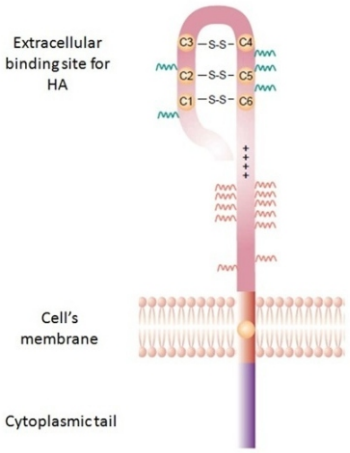

Figure 1. Artistic representation of a CD44 transmembrane receptor.

Technical advancements, potentials and limits in neuroradiology

As anticipated above, HA nanoparticles as carriers for antineoplastic drugs hold the potential for an innovative, high throughput therapeutic approach in neuro-oncological chemotherapy protocols. They are used as innovative nanocarriers in order to provide stability and tolerance to molecules of contrast agents for MRI and other applications.

Hyaluronic acid seems to be used with complex manganese oxide nanoparticles (chemical formula: manganium oxide (Mn_3O_4), Molar mass 228.812g/moL) with potential for selective accumulation within high grade gliomas.

The use of targeted tumor MRI in vivo has been so far mostly tested with polyethylene-glycol. Luo et al. (1999) conjugated Mn_3O_4 with polyethylene-glycol creating nanoparticles of a mean diameter of 8.0nm and characterized by a good water-dispersibility, colloidal stability, cyto-compatibility and hemo-compatibility [16].

Chen et al. (2015) further investigated the applicability of MnO-polyethylene-glycol nanoparticles conjugated with fluorescent dye cyanine5.5 as a dual-model imaging nanoprobe for MRI and for near infrared fluorescence. The dual potential imaging role of those nanoparticles was tested conducting experiments on the detection of brain gliomas in mice, showing both in vivo and ex vivo a preferential accumulation of those nanoprobes in the region of tumor cells [17].

Initial studies on HA nanoparticles are ruling out many of the concerns related not only to drug tolerance, but also those related to the risk of cytotoxicity and genotoxicity which have strongly affected the clinical testing of other drugs with a previously unremarkable laboratory track [18]. For instance, encouraging results in terms of cytotoxicity came from tests conducted on engineered nanoparticles synthesized by the encapsulation of polyethylene-glycol phospholipid shell around the Mn_3O_4 core. By quantifying the induction of reactive oxygen species in human glioblastoma and neuroblastoma cell lines, Choi et al. (2015) demonstrated that the cytotoxicity of these nanoparticles were not significant and confirmed their high potential as an innovative diagnostic tool in CNS tumors [19].

Externalities of HA nanoparticles in Nuclear Medicine

Due to the extraordinary high sensitivity (down to the picomolar level of HA) and to its quantitative nature, radionuclide-based imaging is considered a standard modality for molecular imaging, although burdened by the poor resolution (≈5mm) of both proton emission tomography (PET) and single photon emission tomography (SPET) [20]. According to Lopci et al. (2015), in selected neuro-oncological cases, the use of PET scans can determine a change in treatment management in up to 50% of the cases, and these performances have further supported the optimization of known tracers or the identification of new ones [21]. A major challenge in the field of nuclear medicine is, for example, to develop disease specific nanoprobes with facile and robust radiolabeling strategies for gliomas. The characteristics needed are: imaging stability, improved targeting for elevated efficacy, enhanced sensitivity to detect tumors in their early stages, optimized in vivo pharmacokinetics for reduced nonspecific organs uptake and reduced toxicity. As such, scientists have tried to understand whether the process of HA-coating could be of help in meeting those needs. (Figure 2. Structure of HA and its targets for chemical modification)

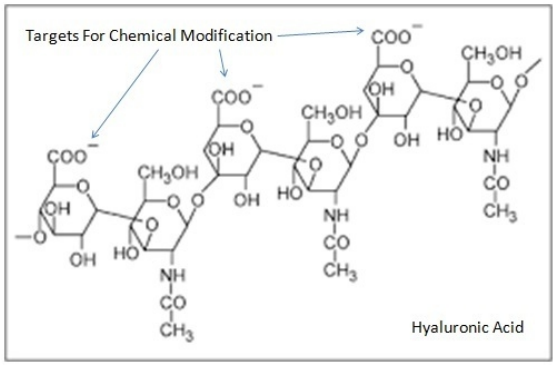

Figure 2. Structure of HA and its targets for chemical modification.

Over the past decade, several positron emitting labeled nanoparticles were developed and substantially improved to meet the diagnostic needs of a wide range of pathological conditions, including inflammatory and oncology ones. Specifically, the rationale behind the attempts to encapsulate radiotracers within HA nanoshells is to leverage on the elective interaction with CD44 and specifically increase the vehiculation within the CNS, while reducing the accumulation elsewhere, and favoring their release in the ECM adjacent to glioma cells. Initial experiments to label HA with indium-11, iodine-125, technetium-99m or carbon-14 (^{14}C) radionuclides date back to the end of last decade [29, 30]. Cozikova et al. (2010) tested in a laboratory setting different

methods for the labeling of HA with routinely used radionuclides aiming to compare the kinetic stability of radiolabeled HA under different conditions (i.e. to mimic the interaction with saline fluids, gastric juice and plasma following intravenous or oral administration); they concluded that the most suitable labeling method may vary from one radionuclide to another, and depends on the specific characteristics of the radiotracer tested.

To date, the main limitation to the HA-coating process has been that the decay of the radiotracers mentioned above is comprised between few minutes and 1 hour, although previous experiments with ^{99}Tc-labeled HA and fluorodeoxyglucose-radiolabeled long-circulating polyethylene-glycol-coated liposomes showed that they could remain in blood circulation at near constant levels for at least 90 minutes [30, 31]. It is well known that the selection of appropriate radionuclides depends on their imaging characteristics, decay half-life, chelating properties, chemistry and availability; for this, the radionuclides selected more recently for conjugation with nanoparticles, such as cuprum-64, bromium-76, zirconium-89 and others who are characterized by longer half-lifes [32, 33].

At present, despite the enthusiasms around HA-coated radiotracers, no one has gone beyond phase II in clinical trials; despite their clinical use is not imminent further investigations are certainly warranted.

In conclusion, the pace of effective translation from bench to bedside of the latest basic science achievements described in this short review article is meant to accelerate even further in the next decade, this will most likely lead to an optimization of the diagnostic tools available for those pathologies, and will potentially support a shift towards personalized medicine for our patients.

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Bibliography

1. Ganau L, Paris M, Ligarotti GK et al. Management of Gliomas: Overview of the Latest Technological Advancements and Related Behavioral Drawbacks. *Behav Neurol* 2015; 2015: 862634.
2. Freed LE, Vunjack-Novakovic G, Biron RJ et al. Biodegradable polymer scaffolds for tissue engineering. *Biotechnology* 1994; 12: 689-93.
3. Ganau M, Prisco L, Pescador D et al. Challenging new targets for CNS-HIV infection. *Front Neurol* 2012; 3: 43.
4. Luo Y, Prestwich GD. Synthesis and selective cytotoxicity of a hyaluronic acid antitumor bioconjugate. *Bioconjug Chem* 1999; 10: 755-63.
5. Ganau M. Tackling gliomas with nanoformulated antineoplastic drugs: suitability of hyaluronic acid nanoparticles. *Clin Transl Oncol* 2014; 16,2: 220-3.
6. Kundu P, Mohanty C, Sahoo SK. Antiglioma activity of curcumin-loaded lipid nanoparticles and its enhanced bioavailability in brain tissue for effective glioblastoma therapy. *Acta Biomater* 2012; 8(7): 2670-87.
7. Madan J, Pandey RS, Jain V et al. A. Poly (ethylene)-glycol conjugated solid lipid nanoparticles of noscapine improve biological half-life, brain delivery and efficacy in glioblastoma cells. *Nanomedicine* 2013; 9(4): 492-503.
8. Tripodo G, Trapani A, Torre ML et al. Hyaluronic acid and its derivatives in drug delivery and imaging: Recent advances and challenges. *Eur J Pharm Biopharm* 2015; 97(Pt B): 400-16.
9. Xin H, Sha X, Jiang X et al. Anti-glioblastoma efficacy and safety of paclitaxel-loading Angiopep-conjugated dual targeting PEG-PCL nanoparticles. *Biomaterials* 2012; 33(32): 8167-76.
10. Fraser JR, Laurent TC, Laurent UB. Hyaluronan: its nature, distribution, functions and turnover. *J Intern Med* 1997; 242(1): 27-33
11. Nitta SK, Numata K. Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. *Int J Mol Sci* 2013; 14(1): 1629-54.
12. Oyarzun-Ampuero FA, Rivera-Rodríguez GR, Alonso MJ et al. Hyaluronan nanocapsules as a new vehicle for intracellular drug delivery. *Eur J Pharm Sci* 2013; 49(4): 483-90.
13. Wohlfart S, Gelperina S, Kreuter J. Transport of drugs across the blood-brain barrier by nanoparticles. *J Control Release* 2012; 161(2): 264-73.
14. Sarkar N, Banerjee J, Hanson AJ et al. Matrix metalloproteinase-assisted triggered release of liposomal contents. *Bioconjug Chem* 2008; 19(1): 57-64
15. Gu G, Xia H, Hu Q et al. PEG-co-PCL nanoparticles modified with MMP-2/9 activatable low molecular weight protamine for enhanced targeted glioblastoma therapy. *Biomaterials* 2013; 34(1): 196-208.
16. Luo Y, Yang J, Li J et al. Facile synthesis and functionalization of manganese oxide nanoparticles for targeted T1-weighted tumor MR imaging. *Colloids Surf. B. Biointerfaces* 2015; 136: 506-13.
17. Chen N, Shao C, Li S et al. Cy5.5 conjugated MnO nanoparticles for magnetic resonance/near-infrared fluorescence dual-modal imaging of brain gliomas. *J Colloid Interface Sci* 2015; 457: 27-34.
18. Greish K, Thiagarajan G, Ghandehari H. In vivo methods of nanotoxicology. *Methods Mol Biol* 2012; 926: 235-53.
19. Choi JS, Choi JY, Na HB et al. Quantitation of Oxidative Stress Gene Expression in Human Cell Lines Treated with Water-Dispersible MnO Nanoparticles. *J Nanosci Nanotechnol* 2015; 15(6): 4126-35.
20. Toy R, Bauer L, Hoimes C et al. Targeted nanotechnology for cancer imaging. *Adv Drug Deliv Rev* 2014; 76: 79-97.
21. Lopci E, Franzese C, Grimaldi M et al. Imaging biomarkers in primary brain tumours. *Eur J Nucl Med Mol Imaging* 2015; 42(4): 597-612.
22. Yamane T, Sakamoto S, Senda M. Clinical impact of ^{11}C-methionine PET on expected management of patients with brain neoplasm. *Eur J Nucl Med Mol Imaging* 2010; 37(4): 685-90.
23. Huang MC, Shih YH, Chen MH et al. Malignancy of intracerebral lesions evaluated with ^{11}C-methionine-PET. *J Clin Neurosci* 2005; 12(7): 775-80.
24. Chung JK, Kim YK, Kim SK et al. Usefulness of ^{11}C-methionine PET in the evaluation of brain lesions that are hypo- or isometabolic on ^{18}F-FDG PET. *Eur J Nucl Med Mol Imaging* 2002; 29(2): 176-82.
25. Shi X, Liu Y, Zhang X et al. The comparison of ^{13}N-ammonia and ^{18}F-FDG in the evaluation of untreated gliomas. *Clin Nucl Med* 2013; 38(7): 522-6
26. Guo Y, Aweda T, Black KC et al. Chemistry and theranostic applications of radiolabeled nanoparticles for cardiovascular, oncological, and pulmonary research. *Curr Top Med Chem* 2013; 13(4): 470-8.
27. Girard N, Courel MN, Véra P et al. Therapeutic efficacy of intralesional ^{131}I-labelled hyaluronectin in grafted human glioblastoma. *Acta Oncol* 2000; 39(1): 81-7.
28. Chakravarty R, Goel S, Dash A et al. Radiolabeled inorganic nanoparticles for positron emission tomography imaging of cancer: an overview. *Q J Nucl Med Mol Imaging* 2017 Jan 26. doi: 10.23736/S1824-4785.17.02969-7. [Epub ahead of print].
29. Cozikova D, Laznickova A, Hermannova M et al. Preparation and the kinetic stability of hyaluronan radiolabeled with ^{111}In, ^{125}I and ^{14}C. *J Pharm Biomed Anal* 2010; 52(4): 517-24 .
30. Marik J, Tartis MS, Zhang H et al. Long-circulating liposomes radiolabeled with [^{18}F]fluorodipalmitin ([^{18}F]FDP) Nucl Med Biol. 2007; 34: 165-7.
31. Laznicek M, Laznickova A, Cozikova D et al. Preclinical pharmaco-kinetics of radiolabelled hyaluronan. Pharmacol Rep. 2012; 64(2): 428-37.
32. Liu Y, Welch MJ. Nanoparticles labeled with positron emitting nuclides: advantages, methods, and applications. *Bioconjug Chem* 2012; 23(4): 671-82.
33. Goel S, England CG, Chen F et al. Positron emission tomography and nanotechnology: A dynamic duo for cancer theranostics. *Adv Drug Deliv Rev*. 2016 Aug 9. pii: S0169-409X(16)30233-2. doi: 10.1016/j.addr.2016.08.001. [Epub ahead of print].