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Comparison of biodistribution profile of monoclonal antibodies nanoparticles and aptamers in rats with breast cancer

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ABSTRACT

The use of monoclonal antibodies and aptamers is growing every single day, as the use of nanoparticle systems. Although most of the products are under investigation, there are a few commercialized products available at the market, for human consume. In this study, we have compared three formulations (aptamer anti-MUC1, monoclonal antibody – Trastuzumab and monoclonal antibodies nanoparticles – PLA/PVA/MMT trastuzumab) to identify their profile as also to understand their behavior into an alive biological system. In this direction the radiolabeling of the products were done and they were all tested in animals (*in vivo*) in two conditions: healthy rats and breast cancer induced animals. The results showed that the nanoparticle has the better biodistribution profile, followed by the aptamer. We conclude that more studies and a global effort to elucidate the biological behavior of drugs and especially nano-drugs are necessary.

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Introduction

Breast cancer is a complex heterogeneous disease and remains the first cause of death by cancer among women worldwide, it presents a great variability on the histopathological and molecular profile (Ferlay et al. 2010, Guiu et al. 2012, Sorlie et al. 2001). The therapeutic regimen of breast cancer can be based on the tumor status of the epidermal growth factor receptor 2 (HER2) (Huober et al. 2010), which is a predictive of treatment responses in trastuzumab (Goldhirsch et al. 2001).

Conventional histopathological characteristics remain relevant to the prognostic of survival (Soerjomataram et al. 2008), however, new methods of diagnosis are searched, in effort to advance diagnosis, prognostic and guide therapy selection.

Trastuzumab is a monoclonal antibody that interferes with the HER2 receptor and it could be used to treat HER2-positive breast cancer (Hudis 2007). This drug enhanced overall survival in the metastatic stage from 20 months to 25 months (Nahta and Esteva 2003). In addition, there are molecules such as aptamers, chemically synthesized oligonucleotides that could recognize peptides and proteins, thus being applicable for therapeutics and diagnostics (Iliuk et al. 2011, Thivyanathan and Gorenstein 2012). Aptamers bind to wide range of relevant proteins with affinities and specificities that are comparable to antibodies (Keefe et al. 2010).

In this sense, polymeric nanoparticles could be used to deliver drugs in cancer therapy and diagnostics. Poly(lactic

acid) (PLA) is a biodegradable polymer widely applied to biological purposes since it is non-toxic and easily eliminated from the body (Liang et al. 2015, Wang et al. 2010).

The present study aimed to investigate and compare the biodistribution profiles of a monoclonal antibody PLA nanoparticle and the aptamer anti-MUC1, both labeled with technetium-99m (^{99m}Tc) in experimental breast cancer model induced in animals.

Study design

In this study, three different formulations were used to test the better option of a radiopharmaceutical for breast cancer imaging. Thus, trastuzumab (Roche Laboratories, Basel, Switzerland), Aptamer Anti-MUC1 (produced by Dr. Sotiris Missailidis Rio de Janeiro, Brazil), and PLA/PVA/MMT trastuzumab nanoparticle were tested in induced animals with breast cancer (three animals for each sample).

Materials

PLA (MW 40–100 kDa) was purchased from Sigma Aldrich (St. Louis, MO). Polyvinyl alcohol (PVA, MW 18 kDa, 85% hydrolyzed), a surfactant, was also purchased from Sigma Aldrich. Trastuzumab was purchased from Roche Laboratories. The aptamer anti-MUC1 was given by Dr. Sotiris Missailidis.

Production of PLA/PVA/MMT/trastuzumab nanoparticles

To formulate the nanoparticles, 50 mg of PLA was dissolved in 2 mL of dichloromethane. Then, this solution was poured into 200 μ L of a 0.1% aqueous solution of a PVA solution (aqueous phase 1), thereby forming a water/oil emulsion. This process was carried out under stirring using a sonicator, and an ice bath was used to prevent heating of the sample and inappropriate premature evaporation of the solvent. Two cycles of sonication (45 W power) for 30 s were used with a 10 s interval between them.

Subsequently, the formed emulsion was then re-emulsified with 4 mL of a 0.7% aqueous solution of PVA and 0.042% clay (second aqueous phase), forming a water/oil/water emulsion. This procedure was performed using two cycles of sonication for 1 min with a 10 s-interval between them. The dichloromethane was evaporated under reduced pressure at room temperature on a rotary evaporator for 20 min. Finally, the nanoparticles were concentrated by ultracentrifugation at 20,000 rpm at 25 °C for 20 min. The nanoparticles were re-suspended in purified water and re-centrifuged twice more in order to remove possible residues of the polymer, surfactant, and organic solvent.

Labeling with Technetium-99 m

Trastuzumab, Aptamer Anti-MUC1, and PLA/PVA/MMT trastuzumab (150 μ L of each) were labeled with technetium-99 m (^{99m}Tc) in an 80 μ g/mL solution of stannous chloride (Sigma Aldrich, St. Louis, MO) for 20 min at room temperature. This mixture was then incubated with a solution of 3.7 MBq (0.3 mL ^{99m}Tc in sodium chloride 0.9%) for 10 min at room temperature.

The quality of labeling process of each single labeling was confirmed by TLC (thin layer chromatography) using acetone as mobile phase (Albernaz et al. 2014, Carvalho Patricio et al. 2013, Leite Diniz et al. 2010).

Induced animals – breast cancer model

Nine female Wistar rats, eight week-old and weighing ~250–300 g were induced with breast cancer. The rats were purchased from Zona Oeste State University (UEZO), Rio de Janeiro, Brazil and the protocols were approved by the Animal Experimentation Ethics Committee – UEZO (Protocol no. 008/14). The animals were maintained under standard conditions of light (12 h light/12 h dark cycle), humidity, temperature, and were fed a standard diet and water *ad libitum*.

For induction of breast cancer, the animals received a single subcutaneous injection of 25 mg/kg DMBA in the breast region (7,12-dimethyl benzo anthracene, Sigma Chemicals) in vehicle (0.5 mL of sunflower oil and 0.5 mL of saline). After 16 weeks, macroscopic and microscopic analyses were performed for tumor confirmation (Di Pasqua et al. 2012, Di Pasqua et al. 2013).

Biodistribution of labeled compounds – pharmacokinetic evaluation

The biodistribution was evaluated in nine female Wistar rats. Three different samples were prepared for injection into

Table 1. Percentage of labeled PLA/PVA/MMT trastuzumab observed over time, after ascending chromatograms of ^{99m}Tc compared with free pertechnetate ($\text{Na}^{99m}\text{TcO}_4^-$)

Time (h)	Labeling (%)
0	99.4 $\pm$ 0.4%
1	99.0 $\pm$ 0.9%
2	98.9 $\pm$ 0.8%
3	98.9 $\pm$ 1.2%
4	98.5 $\pm$ 0.5%

Table 2. Percentage of labeled Aptamer Anti-MUC1 observed over time, after ascending chromatograms of ^{99m}Tc compared with free pertechnetate ($\text{Na}^{99m}\text{TcO}_4^-$)

Time (h)	Labeling (%)
0	98.8 $\pm$ 0.4%
1	98.5 $\pm$ 0.9%
2	97.9 $\pm$ 0.8%
3	98.3 $\pm$ 1.2%
4	98.5 $\pm$ 0.5%

Table 3. Percentage of labeled trastuzumab observed over time, after ascending chromatograms of ^{99m}Tc compared with free pertechnetate ($\text{Na}^{99m}\text{TcO}_4^-$)

Time (h)	Labeling (%)
0	99.2 $\pm$ 0.4%
1	98.7 $\pm$ 0.9%
2	98.9 $\pm$ 0.8%
3	97.3 $\pm$ 1.2%
4	97.5 $\pm$ 0.5%

animals: PLA/PVA/MMT trastuzumab nanoparticles labeled with ^{99m}Tc , Aptamer Anti-MUC1 labeled with ^{99m}Tc , and free trastuzumab labeled with ^{99m}Tc (control). Labeled samples (1.2 MBq per 0.2 mL) were injected by retro-orbital via (Patricio et al. 2015, Wasserman et al. 2014).

Animals were sacrificed after 120 min and their organs were removed and weighed. Radioactivity uptake was assessed using a gamma counter. Results were expressed as dose per organ (%ID/organ). The Institutional Review Board and the Animal Ethics Committee of the Federal University of Pernambuco (no. 23076.002362/2010–37) approved all studies (Cerqueira-Coutinho et al. 2015, Pinto et al. 2014).

Results and discussion

Labeling process

All particles (PLA/PVA/MMT trastuzumab and Aptamer Anti-MUC1) and the free trastuzumab were successfully labeled (>90%) (Tables 1–3).

Induced animals – breast cancer model

After 16 weeks, a tumor was found in the breast region of female rats with a cystic pattern, vascularized and fibrotic (Figure 1A). These lesions were confirmed through the histological analysis, and they display atypical tumor cells rather grouped with hyperchromasia separated by fibrovascular tissue.

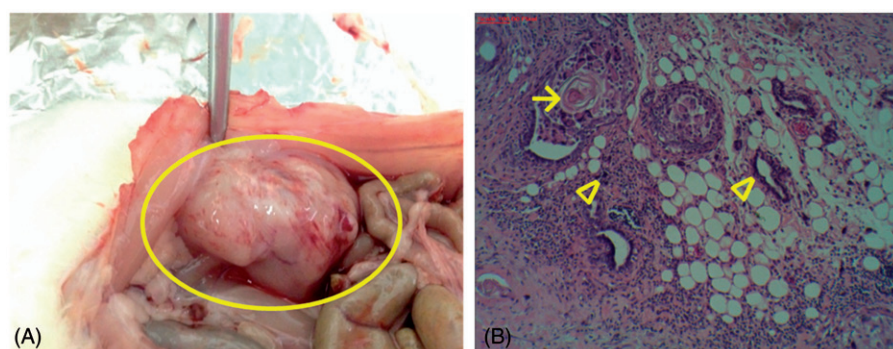


Figure 1. Morphological characteristics of rat mammary carcinoma lesions. (A) Macroscopic observation; (B) histological confirmation revealed by hematoxylin and eosin coloration. Most of the lesions were well vascularized, cystic, and very fibrotic (circle). Histologically, observed area of invasive carcinoma with neoplastic structures with well-stained cells, spreading the mammary stroma and infiltrating the adjacent adipose tissue (arrowheads). The arrow points neoplastic tissue with the presence of squamous cells and keratin deposit. Magnification $\times 200$

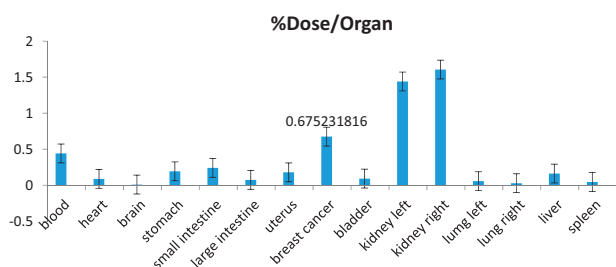


Figure 2. Biodistribution of aptamer anti-MUC1 labeled with ^{99m}Tc in induced animal with breast cancer

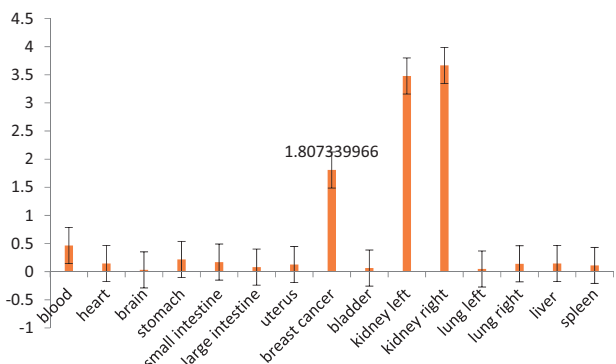


Figure 3. Biodistribution of PLA/PVA/MMT trastuzumab nanoparticles labeled with ^{99m}Tc in induced animal with breast cancer

We also observed neoplastic cells infiltrating the adjacent adipose tissue with the presence of squamous cells and keratin deposit (Rodriguez et al. 2012) (Figure 1B).

Biodistribution

The biodistribution assays were performed and the results are expressed in Figures 2–4.

According to results of biodistribution, it is possible to observe that the higher uptake was associated to the nanoparticle (over the double of the uptake of the aptamer Anti-MUC1 and the free monoclonal antibody, trastuzumab), confirming that nanoparticles have better biodistribution and biodirection than the free drug. It is also important to notice that aptamer Anti-MUC1 has higher affinity than the

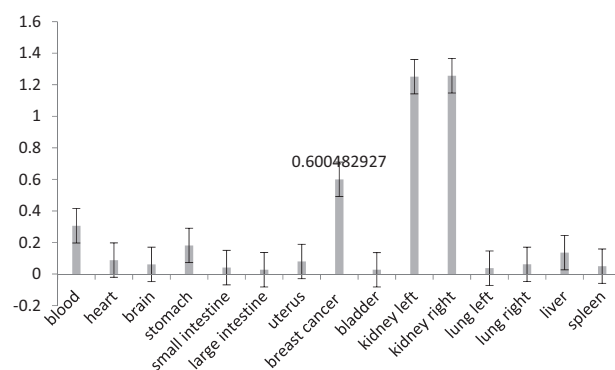


Figure 4. Biodistribution of free trastuzumab labeled with ^{99m}Tc in induced animals with breast cancer

monoclonal antibody, confirming that this class of drug should be more studied, especially to breast cancer. All the three formulations showed an excellent clearance and, subsequently, a low biological half-life of the remaining particles in blood. The presence in blood and heart may be very meaningful because it is normally related to affinity by blood proteins (albumin mostly). In this case, all showed a good affinity. Regarding the recognition by the immunological system, both nanoparticle and aptamer showed the lower levels of recognition which is very desirable on drug delivery system, and corroborates the findings in blood and heart (Carvalho Patricio et al. 2013). The results are excellent for brain uptake, with exception of the free drug. In most cases, only 0.6% of radiopharmaceutical crosses brain barriers, which is required for imaging. In the case of free trastuzumab, 2% crossed the brain barrier and reached the brain, which means that for imaging or therapeutic radiopharmaceutical great areas of the brain (mostly healthy) would receive unnecessary radiation during the examination, which is not recommended. It is also important to notice that although there are many vascular endothelial growth factor (VEGF) receptors in intestine (small and large) the uptake in this region was lower than the cancer region, confirming the over-expression of these ligands on cancer state. Also the nanoparticle was more effective to dribble those receptors than aptamers and free drug, confirming that the drug delivery system (nanoparticle) may be better than the conventional treatment.

Conclusions

The results showed that nanoparticles presented a better bio-distribution than free drugs as well as aptamers in breast cancer model. Also it is possible to think the use of the nanoparticle as a drug delivery system of radiopharmaceuticals for breast cancer imaging.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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