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Characterization and biodistribution of bevacizumab TPGS-based nanomicelles: Preliminary studies

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ABSTRACT

Bevacizumab is an FDA approved monoclonal antibody (anti VEGF) indicated in many cancers, mostly metastatic ones. D-α-tocopheryl polyethylene glycol succinate (TPGS) is the water-soluble form of vitamin E which usually forms micelles. This work aims to report preliminary results of the biodistribution of a TPGS based nano-micelle delivery system for bevacizumab in a gastric cancer xenograft model. Evaluation of the biodistribution of micelles/bevacizumab-99mTc was performed in Balb/c nude mice carrying MKN45 cell line xenograft. The nano-radiopharmaceutical (3.7 MBq/0.2 mL) was administered intraocularly and biodistribution was assesed 1 h post administration. The activity in each organ and blood was determined by a gamma counter. Mean size was 10 ± 1 nm for pure TPGS and 11 ± 1 nm for bevacizumab-TPGS respectively. Biodistribution showed that the highest uptake was found in both lungs and liver. Kidneys had also an important uptake. The tumor accumulated moderate to low radiolabeled nanomicelles, nevertheless tumor/blood ratio was very high.

These preliminary results may help as a start point to continue evaluating the potential of radiolabeled bevacizumab-TPGS based nanomicelles to be used as a theranostic agent.

1. Introduction

Cancer remains one of the most severe life threatening diseases worldwide. The new cases around the world will rise to about 22 million in the next 15 years according to the World Health Organization and Cancer UK research organization [1,2]. From cancer-related deaths, gastric cancer is the third most common cause with carcinoma being the most frequent malignancy [1,3].

Scientific research has made some significant advances in detecting molecules that are highly expressed in tumors or implicated in their growth or progression This has led to the development of specific strategies to target malignant tissue such as the use of monoclonal antibodies [4,5]. Moreover, the incorporation of specific molecules into nano-sized delivery systems rises as an interesting and effective strategy for cancer therapy [6,7].

Bevacizumab is an FDA (Food and Drug Administration) approved monoclonal antibody that reacts against the Vascular Endothelial Growth Factor (VEGF) and is indicated in many cancers, mostly metastatic ones: first-line treatment of metastatic colorectal cancer and of non-small cell lung cancer; second-line treatment of metastatic colorectal cancer and of glioblastoma; metastatic renal cell carcinoma; metastatic Her2-negative breast cancer; metastatic colorectal cancer in combination with fluoropyrimidine-based chemotherapy; persistent, recurrent, or metastatic cervical cancer

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in combination with chemotherapy and platinum-resistant recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer in combination with chemotherapy [8].

Moreover, bevacizumab is being used for the treatment of different pathologies and other types of cancer such as gastric cancer as a combined first line treatment and several biomarkers are being proposed to predict its efficacy [9–12].

D-α-tocopheryl polyethylene glycol succinate (TPGS) is the water-soluble form of vitamin E, resulting from its esterification with polyethylene glycol 1000. Given that it is an amphyphilic compound, it usually forms micelles. Micelles are one of the nano sized particles that can act as drug delivery systems which are particularly useful in oncology given that tumor uptake might be favored by the Enhanced Permeability and Retention effect [17]. TPGS is a Generally Regarded As Safe (GRAS) listed oral supplement which has been approved by the FDA as a safe pharmaceutical adjuvant used in drug formulation and has been investigated for oral and parenteral administration in antineoplastic therapy [13–16].

Delivery of bevacizumab to specific sites of a tumor would enhance its efficacy while ameliorate its adverse effects. Therefore this work aims to report preliminary results of the biodistribution of a TPGS based nano-micelle delivery system for bevacizumab in a gastric cancer xenograft model.

2. Materials and methods

2.1. Nano-micelles preparation

For empty micelles, TPGS (Eastman Chemical Company, USA) was weighted and dissolved in distilled water (10% w/v) with continuous agitation and temperature (30 °C) until a homogeneous dispersion was achieved. After reaching room temperature the solution was used to perform the radiolabeling procedure.

For micelles carrying bevacizumab, antibody (Avastin®) was added to the TPGS dissolving solution while micelles were forming. Bevacizumab concentration in final solution was 2,5 mg/mL.

2.2. Size determination by DLS

Nanoparticles size distribution, mean size and polydispersity index (PDI) of the micelle/bevacizumab nano-system were determined by dynamic light scattering (DLS) using the equipment Zetasizer Nano ZS (Malvern Instruments, UK). Measurements were performed in triplicate at 25 °C and the laser incidence angle in relation to the sample was 173° using a 12 mm^2 quartz cuvette. The mean ± standard deviation (SD) was assessed.

2.3. Tumor xenograft models

MKN45 cells (American Type Culture Collection, Manassas, VALLC) were cultured in RPMI (Gibco, Life technologies, MD, USA) supplemented with 10% of fetal bovine serum (Gibco, Life technologies, MD, USA) and 50 μg/mL of gentamicin (Gibco, Life technologies, MD, USA). Mycoplasma contamination in cultured cells was excluded using Lonza Mycoplasma Detection Kit. For tumor induction, eight-week-old male Balb/c nude mice were implanted subcutaneously with 2×10^6 MKN45 cells and growth was accompanied for 3 weeks. Balb/c nude mice were bred at the animal facility of the Nuclear Energy Research Institute (IPEN) and all experiments were carried out in accordance with EC Directive 86/609/EEC for animal experiments and were approved by local animal ethics committees.

2.4. Labeling process

The method used was the direct labeling process as described previously [18,19]. The labeling process used 150 μL of micelle/bevacizumab nanosystem, which were incubated with stannous chloride ($SnCl_2$) solution (30 μL/mL) (Sigma-Aldrich) for 20 min at room temperature. Then, this solution was incubated with 2 mCi (approximately 300 μL) of technetium-99m (IPEN/CNEN) for 10 min, which labeled the nanomicelles with Tc-99m.

In order to characterize the labeled micelle-bevacizumab nanosystem, thin layer chromatography (TLC) was performed using Whatman paper No 1. The chromatography was carried out by using 2 μl of the labeled-nanomicelles in acetone (Sigma-Aldrich) as mobile phase. The radioactivity of the strips was measured in a gamma counter (Perkin Elmer Wizard® 2470). In order to confirm the efficacy of the labeling process, the chromatography was performed in 2 different times (0 and 3 h).

2.5. Biodistribution

Evaluation of the biodistribution of micelles/bevacizumab-99mTc was performed in 2 Balb/c nude mice which were inducted with the tumor (as previously described) and anaesthetized with 10% Ketamine and 2% Xylazine in 15 μL administered intramuscularly (thigh). The nano-radiopharmaceutical (3.7 MBq/0.2 mL) was administered intraocularly [20]. One hour post administration mice were sacrificed in a carbon dioxide gas chamber and the organs of interest were excised and weighed. The activity in each organ and blood was determined by a gamma counter (Perkin Elmer Wizard® 2470). Results were expressed as injected dose normalized by tissue weight (% ID/g) and as a ratio between the organ and blood pool (tissue/blood) [18]. The following organs were evaluated: brain, right lung, left lung, stomach, spleen, small intestine, large intestine, heart, right kidney, left kidney and liver. Tumor (lesion) uptake was measured as well.

3. Results

Some characteristics of the bevacizumab TPGS based nanomicelles were assessed in vitro. Radiochemical purity resulted higher than 95% at initial time and remained the same post 3 h. Fig. 1 shows size distribution of bevacizumab-TPGS based nanomicelles. Mean size was 10 ± 1 nm for pure TPGS and 11 ± 1 nm for bevacizumab-TPGS. They also presented narrow size distribution and low PDI of 0.22 ± 0.02 and 0.20 ± 0.01 respectively. It is worth mentioning that no statistical differences in size were found when

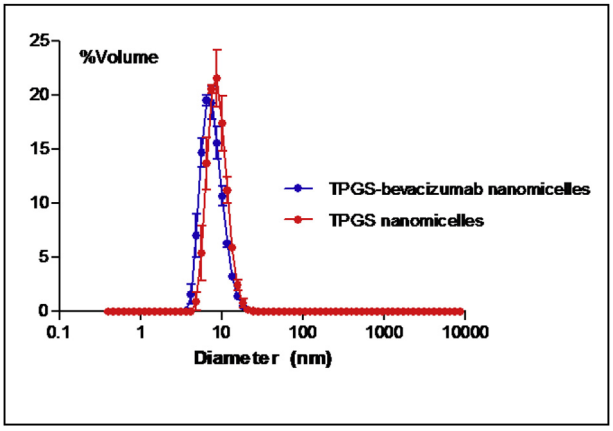

Fig. 1. Size distribution of bevacizumab TPGS-based micelles.

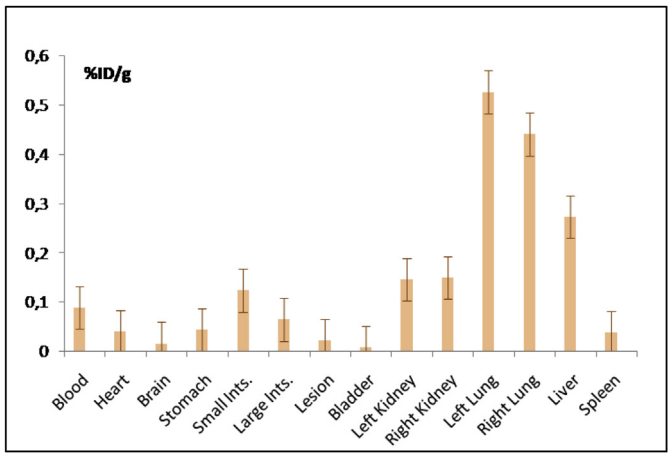

Fig. 2. Biodistribution of bevacizumab TPGS-based micelles in balb/c nude mice with MKN45 cells xenograft. Results of percentage of the injected dose (%ID/g) are shown mean ± SD.

compared to empty TPGS based nanomicelles.

Biological behavior of the nanomicelles was also measured. Results are shown in Fig. 2, where the distribution of bevacizumab-TPGS based nanomicelles is represented. The highest uptake was found in both lungs and liver. Kidneys had also a high uptake showing a possible elimination pathway. The tumor accumulated moderate to low radiolabeled nanomicelles. Nevertheless tumor/blood ratio was very high. This could be seen in Fig. 3.

4. Discussion

Small variations among TPGS nanomicelles size distribution were observed with and without the drug encapsulated. However, the nanomicelles mean size was around 10 nm. In addition, polydispersity index was quite low, indicating homogeneous sizes for all the nanomicelles.

Radiolabeling of the nanomicelles allowed the tracing of this delivery system in an in vivo environment and the localization on specific tissues where it is accumulated in an animal model of gastric cancer. Biodistribution results showed a strong uptake of bevacizumab -TPGS nanomicelles in lung and liver. Given that the nanomicelles have a particle size in the order of 10 nm, liver was an expected target and more specifically the kupffer cells [21]. However, lung accumulation usually involves larger sized particles.

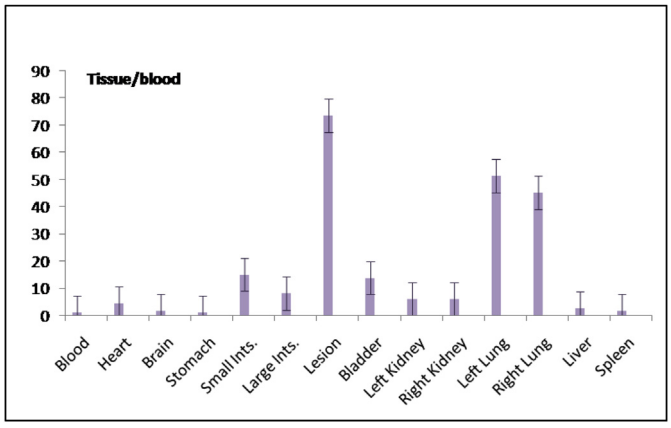

Fig. 3. Biodistribution of bevacizumab TPGS-based micelles. Results of percentage of the injected dose (%ID/g) are shown mean ± SD.

These results might be due to aggregation of nanomicelles in vivo or a specific affinity of these bevacizumab-TPGS nanomicelles for this tissue but further research is needed in order to address this issue. Kidneys and intestine have also high uptake. This might reflect some of the elimination pathways. More time points are needed to elucidate pharmacokinetics and routes of elimination by which bevacizumab -TPGS nanomicelles are excreted.

The tissue/blood ratio for the tumor was very high. This is a promising result for this nano-system to be used as a diagnostic tool in gastric cancer since it could be translated in a high contrast image. It also means that bevacizumab -TPGS nanomicelles have a rapid clearance from blood and after an hour of administration they have been already taken up by different tissues.

It is worth mentioning that the amount of monoclonal antibody administered to the animals was lower than the therapeutic dose reported for humans (5–15 mg/kg) because evaluating the therapeutic effect was not the aim of this work as it was assessing bevacizumab-TPGS based nanomicelles biodistribution. Delivery of therapeutic concentrations of bevacizumab in TPGS based nanomicelles is still an interesting issue that should be addressed by further research in animal models.

Encapsulating and radiolabeling bevacizumab are two strategies that have started to appear in many scientific papers as interesting ways to deliver the antibody to specific target organs and to perform imaging protocols respectively. In many cases the main targets were malignant lesions [22,24]. In others, entrapment bevacizumab has been proposed by investigators for treatment of ocular neovascularization and macular degeneration [25–27]. In this case we propose to combine these strategies and get the best of both approaches.

These preliminary results may help as a start point to continue evaluating the potential of radiolabeled bevacizumab-TPGS based nanomicelles to be used as a theranostic agent. Therefore further research is needed in order to determine its stability and antibody release profile as well as its therapeutic efficacy and imaging quality in tumor bearing animals.

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