Nanoradiopharmaceuticals: Development oF Labeling Process for Polymeric Nanoparticles

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Abstract: Nanomedicine, probably, is the future of modern medicine. Hence, there is a global effort being made in the development of nanopharmaceuticals. Among all the nano-pharmaceuticals developed so far, radiopharmaceuticals are the fewest in number of published studies. The development of nanoradiopharmaceuticals is complex but not impossible. In this work we discuss the possibility and the results of developing 4 nanoradiopharmaceuticals based on 3 different types of nanoparticles as alternative drug delivery systems. Also we present the preliminary results in animals.

Keywords: Radiopharmaceuticals, nanomedicine, nanobiotechnology, drug delivery system.

INTRODUCTION

Polymeric nanoparticles used as drug delivery systems represent a significant development area in the pharmacy field. Both the investment and research have been increasing day by day. The polymeric nanoparticles have great stability, industrial capacity and allows for adjustments to achieve the suitable release profile and /or direction for a particular site of action. The use of poly(lactic-co-glycolic) acid nanoparticles (PLGA NPs) has emerged as a powerful potential methodology for carrying small and large molecules of therapeutic importance as well as scaffolds for tissue engineering applications. Polymeric micelles are used as pharmaceutical carriers to increase solubility and bioavailability of poorly water-soluble drugs. Different ligands have been used to prepare targeted polymeric micelles [1]. Liposomes have a decade-long clinical presence as nanoscale delivery systems. However, their use as delivery systems of nanoparticles is still in the preclinical development stages. Liposome-nanoparticle hybrid constructs present great opportunities in terms of nanoscale delivery system engineering for combinatory therapeutic-imaging modalities. Moreover, many novel materials are being developed in nanotechnology laboratories that often require methodologies to enhance their compatibility with the biological milieu *in vitro* and *in vivo*.

Liposomes are structurally suitable to make nanoparticles biocompatible and offer a clinically proven, versatile platform for the further enhancement of pharmacological efficacy. Small iron oxide nanoparticles, quantum dots, liposomes, silica and polystyrene nanoparticles have been incorporated into liposomes for a variety of different applications [2]. Many methods of labeling liposomes and micelles with both diagnostic and therapeutic radionuclides have been developed since the initial discovery of liposomes 40 years ago. However, successful labelling of such is still in pre-clinical phase. Diagnostic radiolabels can be used to track nanometer-sized liposomes in the body in a quantitative fashion. The same goes for any nanoescale pharmaceutical, such as micelles and microparticles.

The recent developments of nuclear medicine in oncology have involved numerous investigations of novel specific tumor-targeting radiopharmaceuticals as a major area of interest for both cancer imaging and therapy. The current progress in pharmaceutical nanotechnology field has been explored in the design of tumor-targeting nanoscale and microscale carriers that are able to deliver radionuclides in a selective manner to improve the outcome of cancer diagnosis and treatment. These carriers include chiefly liposomes, microparticles, nanoparticles, micelles, dendrimers and hydrogels, among others. Furthermore, combining the more recent nuclear imaging multimodalities which provide high sensitivity and anatomical resolution such as PET/CT (positron emission tomography/computed tomography) and SPECT/CT (combined single photon emission computed tomography/computed tomography system) with the use of these specific tumor-targeting carriers is highly promising and will, hopefully in the near future, allow for earlier tumor detection, better treatment planning and more powerful therapy. In this article we highlight the use, limitations, advantages and possible

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improvements of different nano and microcarriers as potential vehicles for radionuclide delivery in cancer nuclear imaging and radiotherapy [4, 5].

METHODOLOGY

Nanoparticules

Four samples of nanoparticules were analyzed, as follows: samples I and II micelles made up of DSPE-PEG, TGPS and 15% of tamoxifen; sample III nanocapsule of PLA and 15% of tamoxifen and sample IV also a nanocapsule made of PLA-PEG and 15% of tamoxifen. In all the samples the tamoxifen were introduced during the process of production of the nanoparticles. All the samples were donated by the Laboratório de Tecnologia Farmacêutica USP-Ribeirão Preto.

Chromatography

The labeling process was done using 150 µL of (each nanoparticles under study, micelles and nanocapsule, respectively) solution incubated with stannous chloride ($SnCl_2$) solutions (80 µL/mL) (Sigma-Aldrich) for 20 minutes at room temperature. Then this solution was incubated with 100 µCi (approximately 300 µL) of technetium-99m (IPEN/CNEN) for another 10 minutes in order to label their structures with Tc-99m.

In order to characterize the labeled nanoparticles, thin layer chromatography (TLC) was made using Whatman paper nº 1. The TLC was performed using 2 ∫l of each labeled sample in acetone (Proquimios) as mobile phase. The radioactivity of the strips was verified in a gamma counter (Packard, Cobra II) as described in Table **1** and **2**.

Biodistribution

Biodistribution studies [6,7] were done with eight mice, two for each nanoparticle labelled sample (I, II, III and IV). The Institutional Review Board and the Animal Ethics Committee approved the study protocol. The labelled samples (3.7 MBq/0.2 mL) were administered after catheterization of the jugular vein. Planar images were obtained 30 minutes post-injection with a Milennium Gamma Camera (GE Healthcare, Cleveland, USA). Counts were acquired for 5 min in a 15% window centred at 140 KeV. Then, the animals were sacrificed and their organs removed, weighed and the radioactivity uptake counted in a gamma counter (Packard-Cobra II). Results were expressed as percentage of injected dose per gram of tissue in table **3**.

RESULTS AND DISCUSSION

Whatman n°1 chromatography

Results are shown in Tables **1** and **2**.

Table 1: Ascending chromatograms of the ^{99m}Tc-sample I and ^{99m}Tc-sample II compared to free pertechnetate ($Na^{99m}TcO_4^-$)

Samples	Solvent	Bottom (%)	Top (%)
99mTc-sample I	Acetone	80.1	19.9
99mTc-sample II	Acetone	86	14
Na99mTcO4-	Acetone	0.3	99.7

Table 2: Ascending chromatographs of the ^{99m}Tc-sample III and ^{99m}Tc-sample IV compared to $Na^{99m}TcO_4^-$

Samples	Solvent	Bottom (%)	Top (%)
99mTc-sample III	Acetone	92.2	7.8
99mTc-sample IV	Acetone	87.1	12.9
Na99mTcO4-	Acetone	0.3	99.7

All the nanoparticles were successfully labelled (>80%). The use of acetone as mobile phase provided an efficient separation from free Tc-99m and the labelled nanoparticle. In this case the chromatography system can be used as a well-established system for other nanoparticles following the features of the nanoparticles used in this study.

Biodistribution Studies

The results for each labeled sample are below:

The samples I, III and IV shows the liver as the main organ. Sample II, besides the liver, showed the nanoradiopharmaceutical in the blood. It is important to note that none of the nanoparticles crossed the hematoencephalic barrier. Also, samples I, III and IV probably followed the hepatic system, since that the image shows the radiopharmaceuticals principally in the liver after 30 min. This means that their clearance is faster than the sample III that stayed in the blood pool

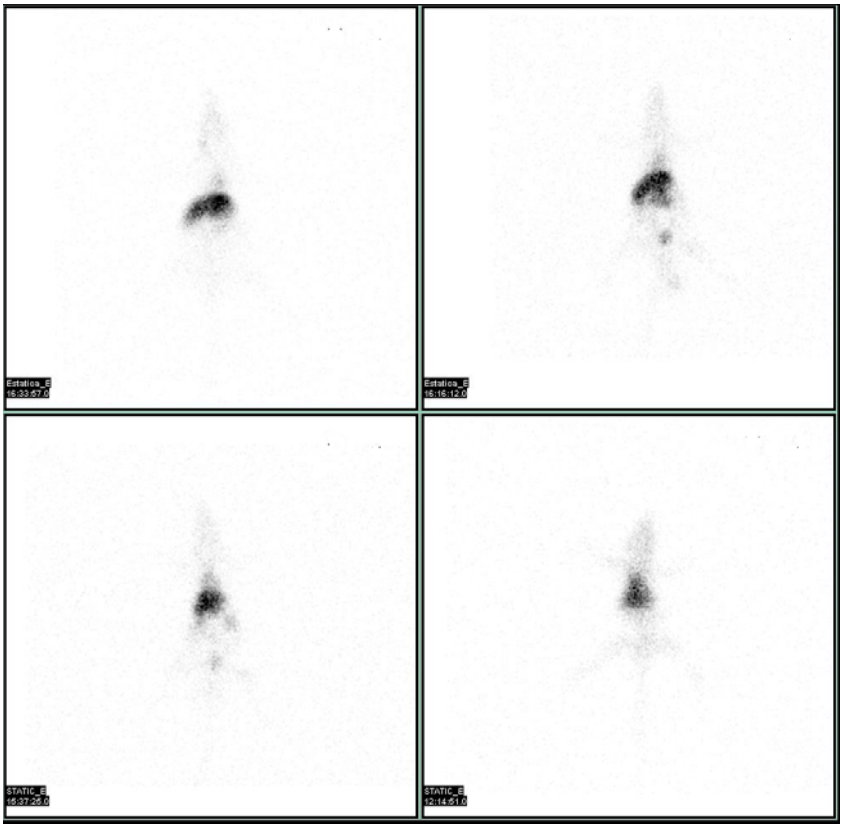

Figure 1: Biodistribution of samples I, II, III and IV in mice.

for a long period, probably due a high affinity for blood proteins.

Table 3: Biodistribution %gram per tissue versus organ of the labeled samples in mice:

Organs	Sample I	Sample II	Sample III	Sample IV
Heart	1.88±0.69	1.17±0.95	0.61±0.43	4.12±0.38
Right lung	2.31±0.43	0.86±0.83	1.04±0.82	4.48±1.52
Left lung	2.29±0.40	0.94±0.91	1.20±0.80	3.06±2.10
Liver	8.06±1.75	3.19±4.33	9.08±2.49	8.41±0.01
Spleen	1.42±0.33	1.43±1.51	2.34±0.25	2.49±0.39
Stomach	0.81±0.40	1.04±0.07	0.21±0.09	1.34±0.77
Intestine	0.46±0.08	1.88±0.96	0.17±0.12	0.92±0.60
Right kidney	8.93±0.86	4.49±6.17	2.72±0.86	8.52±2.37
Left kidney	8.88±1.01	4.50±6.17	2.70±0.96	8.05±2.51

The results of the table **3** are very impressive. The sample I has the one of the highest values of gamma percentage in the kidneys followed by the sample IV which suggest that both of them probably have faster clearance times. These nanoparticles also have a large percentage in the liver, corroborating with the data of figure **1**. The clearance and the high percent of radiopharmaceutical in the liver support the idea that their clearance is a result of their fast metabolism.

Sample III has a higher value in liver, but a low value in kidney. It could be a result of reabsorption before the excretion of the nanoparticle. If it were true the sample III had to be monitored closely for toxicological aspects, given that the nanoparticle is made of tamoxifen.

Nevertheless, sample II demonstrated the strangest behavior. The percentage in the liver is the lowest one which means that the nanoparticle is metabolized slowly. This information is corroborated by the percentage founded in both kidneys, one of the lowest when compared with all the others. The fact that sample III accumulated in the blood pool can bring about unknown consequences related to the metabolism of this nanoparticle. Moreover, further studies must be done in order to evaluate precisely what are the mechanisms involved in this abnormal accumulation of sample III in the blood pool.

CONCLUSION

All of the nanoparticles were successfully labelled with Tc-99m. The consequences are huge since almost 90% of all radiopharmaceuticals are obtained by way of a labelling process. The results, by and large, support the use of this technique to develop nanoradiopharmaceuticals, especially those nanoradiopharmaceuticals based on Tc-99m.

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REFERENCES

[1] Wang T, Petrenko VA, Torchilin VP. Paclitaxel-loaded polymeric micelles modified with mcf-37 cell specific phage protein:enhaced binding to target cancer cells and increased cytotoxicity. Mol Pharmaceutics 2010; 7(4):1007-14.

[2] Al-Jamal W, Al-Jamal K, Bomans P, Frederik P, Kostarelos K. Functionalized-quantum-dot-liposome hybrids as multimodal nanoparticles for cancer. Small 2008; 4(9): 1406-15. http://dx.doi.org/10.1002/smll.200701043

[3] Phillips WT, Goins BA, Bao A Radioactive liposomes. Rev Nanomed Nanobio 2009; 1(1): 69-83.

[4] Hamoudeh M, Kamleh MA, Diab R, Fessi H. Radionuclides delivery systems for nuclear imaging and radiotherapy of cancer. Adv Drug Deliv Rev 2008; 60(12):1329-46. http://dx.doi.org/10.1016/j.addr.2008.04.013

[5] Patrcio BFC, Weissmuller G, Santos-Oliveira R. Development of the 153-Sm-EDTMP nanoradiopharmaceuticals. J Label Compd Radiopharma 2011; 54: S1-S576.

[6] Sa LTM, Albernaz MS, Patricio BFC, Junior MVF, Coelho BF, Bordim A, *et al*. Biodistribution of nanoparticles: initial considerations. J Pharm Biomed Anal 2012; 70: 602-4. http://dx.doi.org/10.1016/j.jpba.2012.06.008

[7] Patrcio BFC, Bordim A, Albernaz MS, Guimaraes TT, Santos-Oliveira R. Influence of radiation on polymeric nanoparticles and in labeling process with 99mTc. Adv Sci Eng Med 2012; 4(3): 271-272(2).

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