Original Article

Development of Nanoradiopharmaceuticals by Labeling Polymer Nanoparticles with Tc-99m

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Abstract

Nanomedicine is considered as the future of modern medicine. Hence, serious global efforts are being made for the development of nanopharmaceuticals. Among all the nanopharmaceuticals developed so far, radiopharmaceuticals constitute only a very small portion, as noted in the published literature. The procedures for development of nanoradiopharmaceuticals are complex. In this paper we discuss the results of a research directed at developing nanoradiopharmaceuticals based on three different types of nanopharmaceuticals as alternative drug delivery systems.

Keywords: Drug delivery system, nanobiotechnology, nanomedicine, radiopharmaceuticals

Introduction

Polymeric microparticles used as drug delivery systems represent a field of significant potential in the field of pharmacy. Overall investment and research activities in this field have been steadily increasing in recent years. The polymeric microparticles have great stability, industrial capacity, and allow 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*.

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Liposomes are structurally suitable to make nanoparticles biocompatible and offer a clinically proven, versatile platform for 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 about 40 years ago. However, their successful labeling 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 nanoscale pharmaceutical, such as micelles and microparticles.[3]

The recent developments of nuclear medicine in oncology have involved numerous investigations of novel specific tumor-targeting radiopharmaceuticals

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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 mostly 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 effective therapy. In this article we highlight the use, limitations, advantages, and possible improvements of different nano and microcarriers as potential vehicles for radionuclide delivery in cancer nuclear imaging and radiotherapy.[4]

Materials and Methods

Nanoparticules

Four samples of nanoparticules were analyzed, as follows: Samples I and II micelles made up of distearoylphosphatidylethanolamine-polyethylene glycol (DSPE-PEG), Tetraglycerol pentastearate (TGPS), and tamoxifen; Sample III nanocapsule of PLA and tamoxifen and Sample IV also a nanocapsule made of poly lactic acid-poly ethylene glycol (PLA-PEG) and tamoxifen. 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 nanoparticle under study, miceles 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 µL of each labeled sample in acetone (Proquimios) as the mobile phase. The radioactivity of the strips was verified in a gamma counter (Packard, Cobra II) as described in Tables 1 and 2.

Biodistribution

Biodistribution studies were done with eight mice, two for each nanoparticle-labeled sample (I, II, III, and IV). The Institutional Review Board and the Animal Ethics Committee approved the study protocol. The labeled 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 minutes 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 [Table 3].

Results

Whatman No. 1 chromatography results are shown in Tables 1 and 2. All the nanoparticles were successfully labeled (>80%). The use of acetone as mobile phase provided an efficient separation from free Tc-99m and the labeled 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.

The results of bio-distribution for each labeled sample are given in Figure 1. Samples I, III, and IV showed liver as

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 (%)
^{99m}Tc-Sample I	Acetone	80.1	19.9
^{99m}Tc-Sample II	Acetone	86	14
Na $^{99m}TcO_4$-	Acetone	0.3	99.7

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

Samples	Solvent	Bottom (%)	Top (%)
^{99m}Tc-Sample III	Acetone	92.2	7.8
^{99m}Tc-Sample IV	Acetone	87.1	12.9
Na $^{99m}TcO_4$-	Acetone	0.3	99.7

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

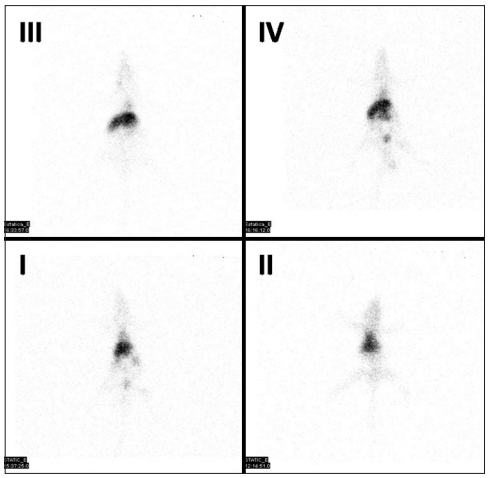

Figure 1: Biodistribution of radiolabeled samples I, II, III, and IV in mice. It may be noted that majority of the radioactivity is seen in the liver in samples I, III, and IV, while Sample II showed predominant retention in the blood pool

the main organ. Sample II showed radiopharmaceutical in the blood pool. It is important to note that none of the nanoparticles crossed the hematoencephalic barrier. Also, Samples I, III, and IV followed the hepatic system which means that their clearance is faster than the Sample III that stayed in the blood pool.

Discussion

The results outlined in Table 3 appear very impressive. Sample I has one of the highest values of counts in the kidneys followed by Sample IV, which means that both of them have faster clearance. These nanoparticles also have a higher percentage uptake in the liver, corroborating the hypothesis 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 due to reabsorption before the clearance of the nanoparticle. If it were true then Sample III has 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 found in both kidneys, also 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 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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