Pharm Res (2017) 34:2922–2930
https://doi.org/10.1007/s11095-017-2275-3

RESEARCH PAPER

Microradiopharmaceutical for Metastatic Melanoma

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Received: 26 August 2017 / Accepted: 1 October 2017 / Published online: 23 October 2017

ABSTRACT

Purpose The purpose of this article was to develop, characterize and test (*in vivo*) dacarbazine microparticles that may be labeled with 99mTc and Ra-223 for both use: diagnostic and therapy of metastatic melanoma.

Methods We developed by double emulsion solvent evaporation methodology the microparticle. The characterization has been done using, Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). The labeling with 99mTc and Ra-223 has been done by the direct labeling process. Also the formulation has been tested pre-clinically using Balb/c mice inducted with melanoma, performing the the biodistribution and planar imaging. Cytotoxicity evaluation was also done in M3 V cell line. In order to understand the safety aspects of the microparticles, microbiological study (endotoxin and sterility) has been done. Finally, planar imaging was performed to evaluate the diagnosing aspect.

Results The results showed that a 559 nm microparticles was obtained with a spherical shape. The labeling process with 99mTc reached over 90% of efficacy. On the other hand, the labeling process with Ra-223 showed a 70% efficacy. The results in inducted animals demonstrated that the microparticles were able to reach the tumor with a high rate (20%). Also demonstrated a low recognition by the Mononuclear Phagocytic System. The cytotoxicity and the microbiological control, corroborates the safety aspect of these microparticles.

Conclusion The planar image and the possible labeling with Ra-223, corroborates the use as a theragnostic agent for imaging and therapy of Metastatic Melanoma.

KEY WORDS cancer · nuclear medicine · microparticles · radiopharmaceuticals

ABBREVIATIONS

99mTc	Technetium 99 metastable
Ac-227	Actinium 227
ALM	Acral Lentiginous Melanoma
Ci	Curie
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle's Medium
DTIC	Dacarbazine
EDTA	Ethylenediamine tetraacetic acid
FBS	Fetal Bovine Serum
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HIL-2	interleukin-2
LAL	Limulus Amebocyte Lysate
LMM	Lentigo Malignant Melanoma
MBq	Mega Becquerel
MM	Metastatic melanoma

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NM	Nodular Melanoma
O/W	Oil/Water
PDI	Polydispersity Index
PLA	Polylactic Acid
PVA	Polyvinyl alcohol
Ra-223	Radium 223
SSM	Superficial Spreading Melanoma
WHO	World Health Organization

INTRODUCTION

The number of cancer worldwide is increasing every year. Cancer is responsible for more than 12% of all causes of death worldwide, with an annual death rate of more than 7 million people (1,2). In this scenario melanoma is is responsible for approximately 132.000 each year (3). The incidence of metastatic melanoma (MM) has more than doubled since 1973, and although considered the least common type of skin cancer (accounting for only about 1% of all cases) is the most deadly, responsible for the vast majority of skin cancer death. Only in USA, in 2016, was estimated that almost 76,380 new cases of melanoma and 10,130 deaths had happened (4–6).

The metastatic melanoma is a tumor of neuroectodermal origin. Is originated from melanocytes in turn, migrating from the neural crest to the epidermis during embryogenesis. When these cells undergo neoplastic transformation they become malignant, originating the melanoma (7–9). Its pathogenesis is not yet fully understood and there are four main subtypes of melanoma: lentigo malignant melanoma (LMM), superficial spreading melanoma (SSM), acral lentiginous melanoma (ALM), and nodular melanoma (NM). In all this cases the tumorigenesis involves a series of poorly understood allelic deletions at several chromosomes, including 1p, 6q, 9p or 10q, 11q, and 17q (10–14) . Is important to notice that i n advanced stages the death rate is considerably high due the lack of effective treatment options. In numbers, it means that 95% of the patients diagnosed with MM will die in last than 5 year (15,16).

There are few therapeutic alternatives for the treatment of MM and the only drugs approved by the Food and Drug Administration (FDA) until 2012 were Dacarbazine (DTIC) and high doses of interleukin-2 (HIL-2). Both with low response rates, between 10–20% (17–22).

In this direction and in order to avoid this critical scenario, the early and properly diagnosing of metastatic melanoma is the main key for its potential cure. For thist reason, in this study we developed and tested a new microradiopharmaceuticals (micro-Dacarbazine labelled with 99mTc and Ra-223) for metastatic melanoma imaging and therapy.

MATERIALS AND METHODS

Development of Dacarbazine Microparticles

To the microparticles preparation, an amount of 5 mg of dacarbazine was weighted (which represents 10% of the polymer mass to be added to the microparticle) and solubilized in 1 mL 0.1 wt% PVA (solution A). Dacarbazine microparticles were prepared by double emulsion solvent evaporation methodology. For this procedure, 1 mL of solution A was dripped into 3 mL of dichloromethane, where 50 mg of PLA (with a molar mass of 60,000 g/mol) were previously solubilized. The mixture was processed using ultra-turrax for 2 min at 12,000 rpm to produce a water-in-oil (O/W) emulsion. This emulsion was emulsified again with 6 mL of PVA 1 wt% solution by ultra-turrax processing for 2 min (12,000 rpm) to produce a W/O/W emulsion. In addition, empty PLA microparticles were also prepared applying the same methodology. Then dichloromethane was evaporated under reduced pressure during 1 h at 25°C. PLA-dacarbazine microparticles and PLA microparticles were recovered by centrifugation (20,000 rpm for 20 min) and washed twice with distilled water to remove the excess of PVA.

Size Determination by Dynamic Light Scattering

Microparticles size distribution, mean size and polydispersity index (PDI) 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.

Scanning Electron Microscopy (SEM)

The morphology of microparticles were examined by Scanning Electron Microscopy (SEM) (TM 3000 – Hitachi). The nanostructures and size distribution of the synthetic nanoparticles were examined by Scanning Electron Microscopy (SEM) using a TM 3000 Hitachi (Hitachi Co, Tokyo, Japan) operating at an acceleration voltage of 10 kV. The dacarbazine microparticles for SEM investigations were prepared by placing 10 µL of nanoparticle suspension on a carbon strip allowing this to dry in air under sterile conditions.

Labelling with 99mTechenitium

The method used was the direct labelling process as described by Pinto *et al.* (23). A fresh solution of 99mTc 100 µCi (approximately 300 µL) was incubated with stannous chloride ($SnCl_2$) solution (30 µL/mL) (Sigma-Aldrich) for 20 min at room temperature. Then, 150 µL of each compound (empty

microparticle, loaded microparticle with dacarbazine and free dacarbazine), were incubated with the 99mTc reduced solution for another 10 min.

Quality Control of the Labeling Process

In order to characterize the labeled compounds (empty microparticle, loaded microparticle with dacarbazine and free dacarbazine), paper chromatography was made using Whatman paper n° 1. The paper chromatography was performed using 2 μL of the labeled-compound, and acetone (Sigma-Aldrich) as mobile phase. The radioactivity of the strips was verified in a gamma counter (Perkin Elmer Wizard® 2470, Shelton, CT City, State).

Stability

In order to characterize the stability of the labeled microparticle loaded with dacarbazine, paper chromatography was made using Whatman paper n° 1 in 5 different times (0,1,2,4 and 6 h). Before each performance of paper chromatography the labeled microparticle were left in a solution of NaCl (0,9%) by the time expressed above. The paper chromatography was performed using 2 μL of the labeled-microparticle and acetone (Sigma-Aldrich) as mobile phase. The radioactivity of the strips was verified in a gamma counter (Perkin Elmer Wizard® 2470, Shelton, CT City, State).

Labeling with Radium 223

Preparation of 223Ra Stock Solution

Aged 227Ac source in equilibrium with its decay products was used for the preparation of 223Ra stock (Amersham, UK), using the Dowex-1 9 8 resin (Sigma-Aldrich, Czech Republic) (24). Shortly, the vial with dry 227Ac source was washed with 600 μL of 1 M HNO3 and the obtained 116 solution was mixed with 3.4 mL of methanol and loaded on a 10 cm glass disposable column (Sigma-Aldrich, Czech republic) loaded with approx. 2 g (wet weight) of Dowex-1 9 8 resin. The gravitational force elution of 223 Ra was performed with a mixture of 0.7 M HNO3 and 80% methanol. The collected eluate was evaporated to dryness on a rotary vacuum evaporator and reconstituted in 1 mL of 1 M HNO3. The 227Ac/223Ra generator was milked every 2 weeks. The final 223Ra stock solution activity was approximately 1 MBq (aV 126 = 1 MBq/mL).

Preparation of Ra-223 Labeled Dacarbazine Microparticles

Approximately 300 μL Ra-223 stock solution (pH adjusted to 10 with aqueous ammonia) was added to 300 μL of dacarbazine microparticles and reaction mixtures were vortex-shaken during 1 h at laboratory temperature. 223Ra-dacarbazine microparticles were centrifuged (20 min at 5000 rpm speed) and the supernatant was removed. 223Ra- dacarbazine microparticle were washed again with ultrapure water and finally left in 500 μL water suspension.

In Vivo Analysis

Tumor Xenograft Models

SK-MEL-37 cells (Memorial Sloan-Kettering Cancer Center, N. Y., USA) 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.

Tumors were established by subcutaneous (sc) injection of $1x10^5$ SK-MEL-37 cells at the right flank of eight-week-old female Balb/c nude mice. Tumor size was monitored for 3 weeks and measured by a caliper. Mice were observed three times per week for evidence of distress, ascites, paralysis or excessive weight loss.

Biodistribution Studies

Evaluation of the biodistribution (Fig. 1) was made with 3 groups using healthy mice: a) Control Group using empty microparticles ($n = 6$), b) Intervention Group using loaded microparticles with dacarbazine (n = 6) and c) Positive Control Group using free dacarbazine (n = 6), and 1 group using inducted mice with metastatic melanoma: a) Microparticle Loaded with Dacarbazine as described in Fig. 1. All labeled with 99mTc. Mice were anesthetized with mix solution of 10% Ketamine and 2% Xylazine in volume of 15 μL and administered intramuscularly (thigh). The 4 compounds (3.7 MBq in volume of 0.2 mL) were administered by retro-orbital via. All the groups were sacrificed by asphyxiation using a carbon dioxide gas chamber after two hours (120 min) of radiocompound administration. Organs (brain, lungs, kidneys, stomach, small and large intestine, bladder, heart, blood pool and tumor) were removed, weighted and the activity in each organ and blood was counted by a gamma counter (Perkin Elmer Wizard® 2470). The results were expressed as uCiper organ. This study and the animal procedures were approved by the University of Pernambuco Ethics Committee, under the number: 23,076,020,578,201,327. All animal experiments were done in accordance with the regulations and guidelines

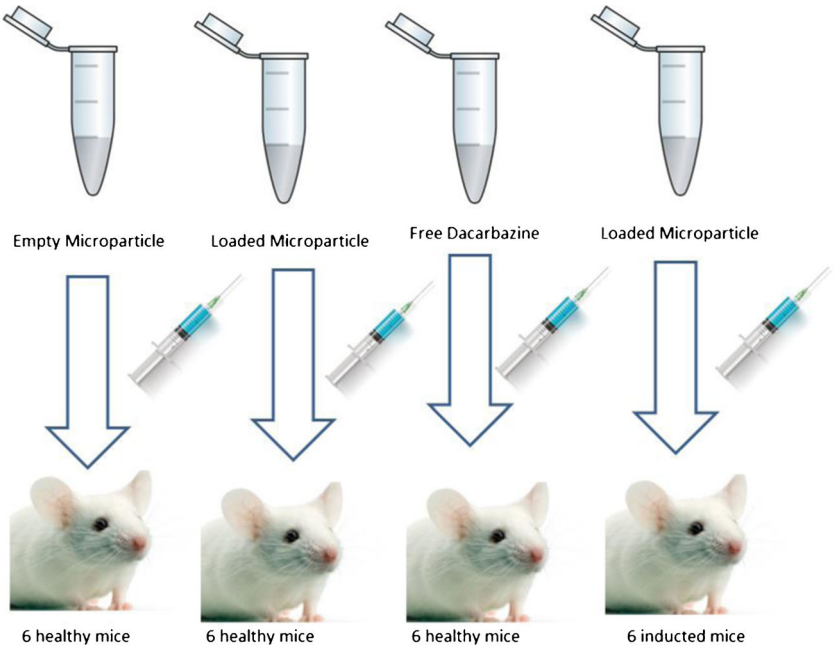

Fig. 1 Schematic Experimental Design

of Brazilian Law for animal experiments (Law number 11.794/2008 and Decree 6.899/2009).

Planar Imaging

Planar images were obtained 90 min after retro-orbital injection of the ^{99m}Tc-Dacarbazine microparticles (2.7 MBq in 0.3 mL)in one mice, integrating for 5 min radiation counts centered at 140 KeV, with a Millenium Gamma Camera (GE Healthcare, Cleveland, USA),using a 15% window.

Cytotoxicity Assay

MV3 human melanoma cells, obtained from Dr. C. Marcienkewicz (Temple University Center for Neurovirology and Cancer Biology, PA, USA), were cultured in DMEM, enriched with 10% FBS, 3.7 g/L sodium bicarbonate, 5.2 g/L HEPES, 0.5 U/mL penicillin, and 0.5 mg/mL streptomycin at 37oC/ 5% CO2. After reaching confluence, cells were detached by a brief treatment with 0.1%/0.01% trypsin/EDTA, collected by centrifugation, re-suspended in fresh 10% FBS medium DMEM and cultured (104 cells/well) on 96-well flat plates, overnight. After that, cells were treated with microparticles loaded with Dacarbazine (0.1–10 μg/mL) in fresh 1% FBS medium DMEM, at 37°C in humidified 5% CO2. After 72 h, MTT assay was performed as previously described (25). Briefly, cells were incubated with MTT (1 mg/ ml) in 1% FBS DMEM, in the dark at 37°C, for 2 h, allowing MTT be reduced to formazan crystals by viable cells. The formazan crystals formed were dissolved in isopropanol for 30 min and the optical densitometry obtained using a microplate reader (BIO-RAD) with 570 nm filter. For calculation, a standard curve was built using increasing concentrations of adhered MV3 cells (103–5 x 104 cells/ well) cultured overnight at 37oC in 5% CO2 atmosphere, to perform The MTT assay as described. Results are shown as percentage of control, of two independent experiments performed in triplicate.

Bacterial Endotoxins Test

Water for injection; *Escherichia coli* standard endotoxin (Endosafe TM, lot N0: EX 64062); disposable polypropylene pipet tips; LAL reagent (Limulus Amebocyte Lysate) (Endosafe TM, lot N0: H1901L); pyrogen-free test tubes (10 mm x 75 mm; 18 mm x 150 mm); graduated pipettes; automatic pipette (Brand®, Transferpette); Minishaker (Ika-Works®); drying oven (Nova Ética); heated ultrasonic bath (Ultrasonic Cleaner, Unique).

All materials used were submitted to dry heat (250° C). The LAL reagent and the endotoxin standard lyophilized were stored in cold temperatures between 2 and 8° C, according the label before use. The LAL reagent was solubilized in water for injection, according to the manufacturer's specifications. Serial dilutions prior to use confirmed a LAL clot sensitivity of 0.125 EU/mL. Standard endotoxin solution was prepared according with the specifications in the label preparation, using water for injection as a diluent.

Samples of dacarbazine microparticles were prepared in water for injectable and serial dilutions have been carried out using the same solvent to obtain 0.52 EU/ mL.

The gel Clot LAL test was conducted in duplicate in accordance to the General Chapter <85> Bacterial Endotoxins Test of the United States Pharmacopeia (USP 39).

Sterility Test

Trypticase soy broth (TSB) (DifcoTM); trypticase soy agar (TSA) (DifcoTM); trypticase soy agar (DifcoTM); thioglycollate medium (OxoidTM); petri dishes (Interlab TM); laminar flow cabinet (Veco do Brasil Ind. Com. Equipamentos Ltda®; Biosafe Plus Classe II Tipo B2); autoclave (Sercon®; HSI 0101); pH-meter (Micronal®, B474); colony counter (Phoenix®, CP600 Plus); automatic pipette (MLA®); laboratory incubator (Fanem®; 347-CD); semi-analytical balance (Gehaka®; BG 1000).

Direct inoculation method described in the United States Pharmacopeia, General Chapter <71> Sterility tests, was applied. The dacarbazine microparticle samples were directly transferred aseptically into media to access presence of any viable organism. The test was performed in duplicate.

RESULTS AND DISCUSSION

Dacarbazine Microparticle Mean Size Assessment

Dacarbazine MPs presented a mean size of 559 ± 11.5 nm, with a PDI of 0.18 ± 0.04 showing homogeneous size for the microparticle (Fig. 2). The use of polymer microparticle as a tool for drug delivery was based in the property of acting as depots, leading to the slow release of the drug and thereby enhancing the efficacy of the treatment. It is also important that the polymer biodegradability and biocompatibility with the body, avoiding cytotoxicity was planned. The low value of PDI corroborates the homogeneity of the microparticles.

Scanning Electron Microscopy (SEM)

The SEM analysis corroborated the DLS findings and confirmed the morphology of the microparticles, as spherical. Considering the average diameter of the microparticles (559 nm) which corresponds to a volume of 0,0351μm^3, assuming the spherical shape of the microparticle, the circumference eq. ($4/3\pi r^3$) and the density of the PLA (1.430 g·cm^{-3}), we estimated that the weight of one single microparticle was about 50.10^{-15} g. Hence, we assumed that in 1 mg of the solution of the dacarbazine microparticles, we have 10^{12-13} dacarbazine microparticles (Figs. 3 and 4).

Labelling with 99mTc

All the compounds (free dacarbazine, empty microparticle and loaded microparticles with dacarbazine) were successfully labeled with 99mTc. The average of labeling efficacy was over 99% in all cases (Table 1).

Stability

The stability of the labeling process from the dacarbazine microparticle with the 99mTc was checked and the values are expressed in Table 2.

Is possible to observe that after 6 h the 99mTc is still labeled with the dacarbazine microparticle, corroborating its stability.

Labeling with 223Ra

Although the results obtained from Ra-223 showed the possibility of labeling with this radionuclide for therapy (70% yield radiolabeling), the low stability of this labeling process (after 3 washes the radiocomplex reduced this value to almost 50%) suggesting that structural modifications of the dacarbazine-microparticles would be needed for stable therapeutically nuclide labeling. But is a promising field that will be exploited in future studies.

Biodistribution

The biodistribution in healthy mice is showed in Fig. 5. In order to compare and understand the behavior of the dacarbazine microparticles in a biological system, was also made the biodistribution in healthy mice of the empty microparticles and the free drug (dacarbazine solely), all labeled with 99mTc.

In all the cases the percentage uptakes in brain were negligible. The empty microparticles showed a very low uptake by the stomach, however the uptake of the loaded and the free drug were very high, this occur due the fact that dacarbazine is

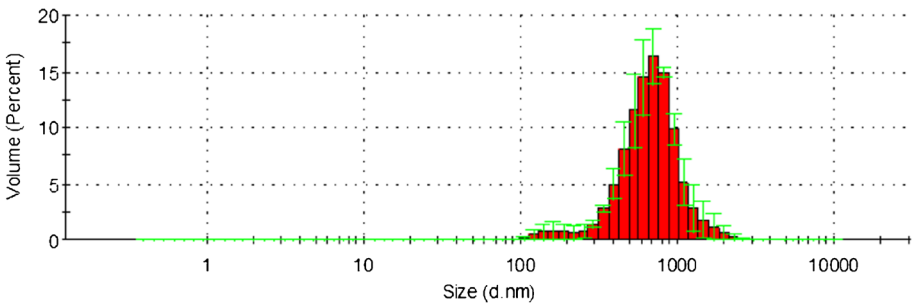

Fig. 2 Dacarbazide MPs mean size and size distribution

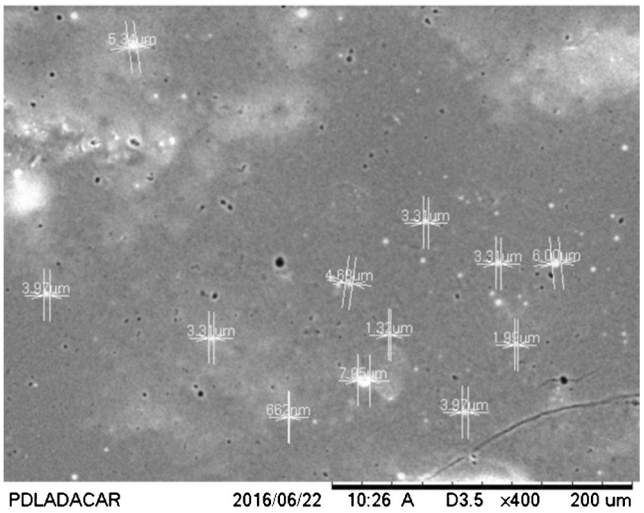

Fig. 3 Scanning Electron Microscopy (SEM) performed in a Hitachi 3000 equipment showing the different sizes of the microparticles of dacarbazine formed during the microencapsulation

a cell-cycle nonspecific chemotherapy drug, classified as an alkylating agent, acting most efficiently in cells that are rapidly dividing. Unfortunately, chemotherapeutics classified as cell-cycle non-specific do not know the difference between the cancerous cells and the normal cells. The "normal" cells most commonly affected by these drugs are blood cells, the cells in the mouth, stomach and the gastrointestinal tract and bowel, and the hair follicles, as stated by the law of Bergonie Tribondeau. This explains the high presence in stomach and intestine of the microparticles loaded with dacarbazine and the free dacarbazine when compared with the empty microparticles.

Is important to notice that the presence of polymer does not interfere in this result, since the uptake of the empty

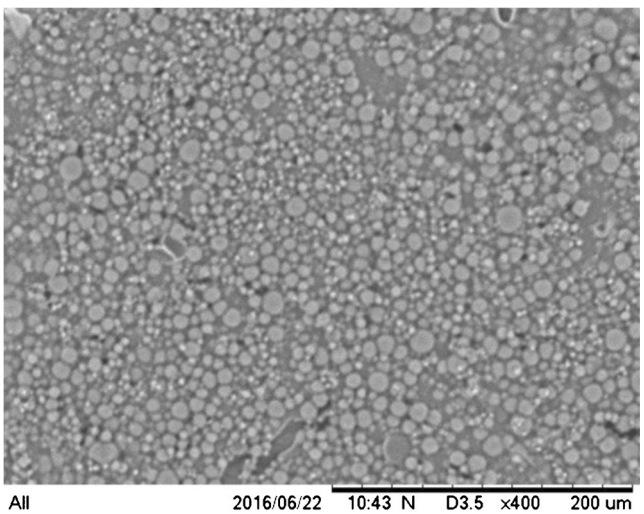

Fig. 4 Scanning Electron Microscopy (SEM) showing overview of the microparticles formed after microencapsulation of the dacarbazine and the most frequent size of microparticles in a range of 540 nm

Table 1 Efficacy of the Labeling process with 99mTc Free Dacarbazine, Empty Microparticle and Loaded microparticles with Dacarbazine

Compound	Efficacy of labeling at 6 h
Free Dacarbazine	99,54 ± 0,5
Empty Microparticle	99,85 ± 0,7
Loaded Microparticle with Dacarbazine	99,76 ± 0,9

microparticles is very low in intestine and stomach. The high presence of the empty microparticles in the kidneys cannot be well explained, however it has been observed before by Steinbacher *et al.* (26).

Is possible to observe that dacarbazine microparticles showed a low uptake by the kidneys when comparing with the free dacarbazine and the empty microparticles. This result may be explained by two theories: 1- The loaded microparticles may show a different excretion route (enterohepatic via) and 2- the loaded microparticles due to its size and affinity for blood constituents may reach the GI tract easier than the free dacarbazine or the empty microparticles (27). The first theory seems to be confirmed by the result from the empty microparticles biodistribution. So, the most probable explanation is that the loaded microparticles may reach the GI tract easier.

An important data is the findings in blood, where the dacarbazine microparticles showed a high uptake, meaning that the drug delivery has a high affinity for blood proteins especially the albumin. This is quite desirable once one of the main goals in the use of the microparticles is the long-lasting circulating delivery system, which has been achieved in this case. Is also important to notice that the uptake of the microparticles loaded with dacarbazine by liver and spleen were lower than the free dacarbazine, and consequently the rapid clearance by the mononuclear phagocytic system (MPS) was avoided, as desirable for drug delivery system based on nanotechnology.

The biodistribution in inducted animals (Fig. 6) showed that the loaded microparticles were able to reach the metastatic melanoma (lesion) with a total dose of Σ 0,019uCi (20%) of the total dose administered. This amount is more than sufficient to perform an SPECT (Single Photon Emission Computed Tomography) and may be a non-invasive, alternative method for diagnosing metastatic melanoma. Also, the

Table 2 Percentage of Labeled Microparticlesafter Ascending chromatograms of ^{99m}Tc Compared with Free Pertechnetate ($Na^{99m}TcO_4^-$)

Time (h)	Labeling (%)
0	99,76 ± 0,9
1	99.4 ± 0.9
2	99 ± 0.8
4	98.9 ± 0.5
6	99.5 ± 0.7

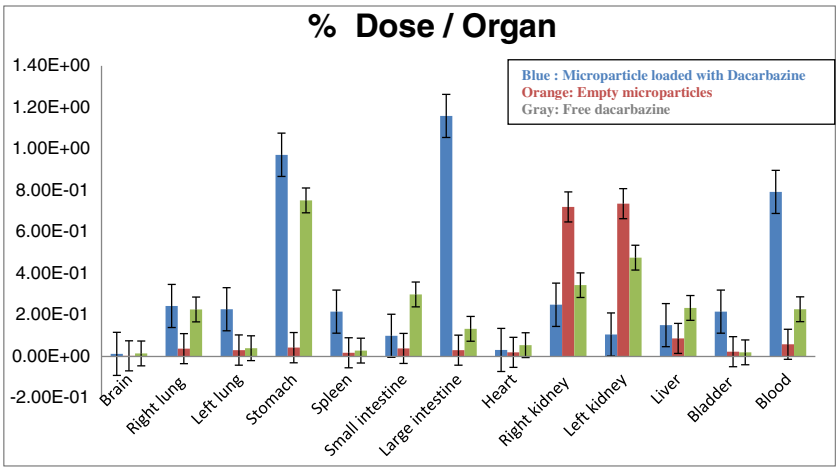

Fig. 5 Biodistribution of the compounds in healthy mice in order to compare with the inducted mice and better understand the biological behavior. In blue is the biodistribution of the dacarbazine microparticle, in red the empty microparticle and in green the free dacarbazine

total amount of loaded microparticles that reached the metastatic melanoma confirms that loaded microparticles labeled with Ra-223 may be used as therapeutic carrier for alpha-radiotherapy using microradiopharmaceutcal.

The results confirmed the high uptake by the intestine (small and large) and stomach. In this case, the possible explanation is the excretion via and the cell-cycle non-specific mechanism (both explained above). Another explanation may be given by Liang *et al.* (28). According to the authors, differently of the healthy mice in the inducted mice with malignant melanoma early and impalpable metastases into the gastrointestinal (GI) tract may be the responsible for the uptake, considering that GI tract is the most common site for metastases. The uptake by the liver was slightly higher than in the healthy mice. This is explained by the fact that during a cancer disease all the hepatic function is highly activated and as a consequence the uptake may be also very high. Nevertheless the size of the microparticles also facilitates its uptake by the liver. However, the low uptake by the spleen confirms the non recognition by the MPS (mononuclear phagocytic system). The high presence of microparticles in blood corroborates that the microparticles must have a high affinity by the albumin, increasing the circulating time. Is also important to notice that the uptake by the brain remains negligible and the renal clearance was the same as in the healthy animals corroborating that due the size these microparticles should have enterohepatic excretion.

The data from planar image (Fig. 7) demonstrated the potential use of the microparticles for imaging melanoma in inducted animals.

Cytotoxicity

The results of the cytotoxicity in M3 V cells (Fig. 7) showed that the Microparticle loaded with Dacarbazine have non-cytotoxicity effect. This result was expected since the amount of dacarbazine used for the production of microparticle was much lower (less than 10% of the therapeutic dose). So, the observed effect will be exclusively due to the microparticle-associated radionuclides and not due the microparticle itself (Fig. 8).

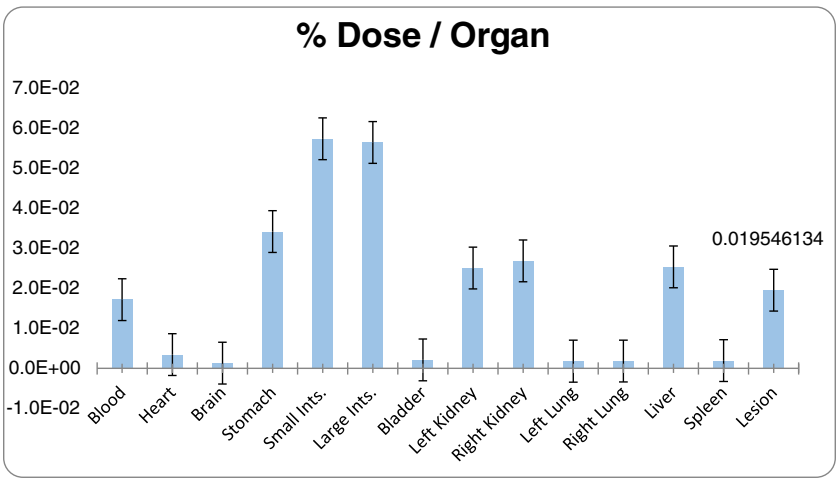

Fig. 6 The biodistribution in inducted mice of the microparticles loaded with dacarbazine

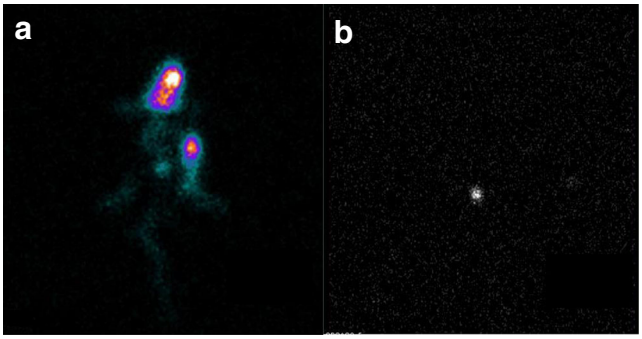

Fig. 7 **a** Picture of the inducted mice showing the tumor growth in the mouse right thigh muscle. **b** Planar image of the whole body of the mice and **c** planar image of the excised lesion showing the regular uptake of the labeled microparticles.

Bacterial Endotoxins Test

After the incubation time, no growth was observed, as expressed in Fig. 9.

Sterility Test

The result was negative for bacterial growth as expressed in Fig. 10.

In both cases the production under pharmaceutical conditions and the labeling process did not contaminate the formulation. These results confirm the possibility of use this methodology to produce microradiopharmaceutical as kit for in house labeling at radiopharmacies.

Conclusion

The results showed that the dacarbazine microparticles may be used as SPECT microradiopharmaceutical labeled with 99mTc for diagnosing metastatic melanoma as an alternative, noninvasive technique. Also showed the possibility to label with Ra-223, endowing this microparticles of therapeutic properties, especially for alpha-therapy. The safety of this

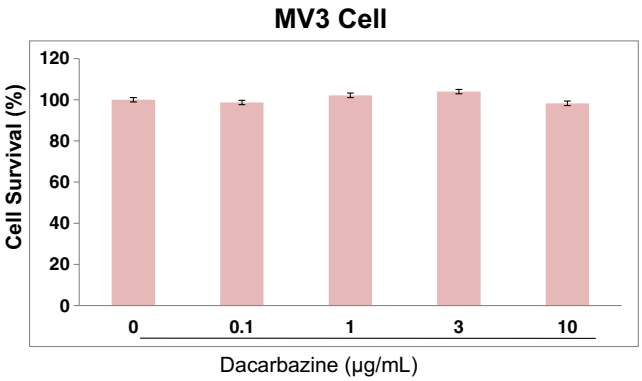

Fig. 8 Cytotoxicity result of the microparticles loaded with dacarbazine, showing the safety for use

	λ (0.125 EU/mL)	
	0 EU/mL	0.52 EU/ml
Dacarbazine Microparticle		
Positive Control		
Negative Control		

Positive control was microbiological solution with E. coli

Negative Control: sterile water

Legend: Green: no clot formation and Red: clot formation

Fig. 9 Limulus Amebocyte Lysate result from the Dacarbazine microparticle. Positive control was microbiological solution with *E. coli*. Negative Control: sterile water. Legend: Green: no clot formation and Red: clot formation

microradiopharmaceutical was corroborated by the cytotoxicity and the microbiological test. In the view of all data collected in this study we believe that this microparticle is a promising theragnostic agent for mestastatic melanoma.

ACKNOWLEDGEMENTS AND DISCLOSURE

The author(s) declare no competing financial interests. The authors would like to thank the National Scientific and Technological Research Council (CNPQ) and the Rio de Janeiro State Research Foundation (FAPERJ) for funding. All animal experiments were done in accordance with the regulations and guidelines of Brazilian Law for animal experiments (Law number 11.794/2008 and Decree 6.899/2009). No applicable.

AUTHOR CONTRIBUTIONS

Ralph Santos-Oliveira: Conceptualization, methodology, validation, investigation, supervision, and resources. Thiago Goulart Rosa, Sofia Nascimento dos Santos: writing–original draft, writing–review and editing, visualization. Terezinha de Jesus Andreoli Pinto, Daniele Molim Ghisleni, Thereza Christina Barja-Fidalgo and Eduardo Ricci, Mohamed Al-Qhatani, Jan Kozempel and Emerson Soares Bernardes: methodology, investigation, resources, writing–review and

Fig. 10 Sterility test image showing no growth of bacteria

editing, and project administration and data curation.

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