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RESEARCH PAPER

Synthesis, characterization and investigation of biodistribution of dendrimer-amiloride nanoconjugate using single photon computed tomography technique in animal sample

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ABSTRACT

Objective(s): Amiloride is a pyrazine compound that inhibits the reabsorption of sodium by blocking sodium channels in the cells of the renal cortex. It has demonstrated promising efficacy in the treatment of cancer in recent times. This study assessed the *in vivo* biodistribution of amiloride conjugated to dendrimer as a targeted agent utilizing SPECT imaging.

Materials and Methods: The dendrimer was synthesised using polyethylene glycol and citric acid as precursors, and dicyclohexyl carbodiimide as a zero-order crosslinker. Amiloride was then conjugated to the dendrimer through the terminal amine group, forming an amide bond with the acidic group of the dendrimer. The synthetic particles were assessed by characterization techniques including FTIR, TEM, LC-Mass, and MAP. The response surface optimization method based on the core chemical was employed to achieve maximum labelling efficiency. The ideal circumstances and biodistribution in the *in vivo* environment were assessed.

Results: TThe characterization findings demonstrated the effective formation and linkage of the nanoconjugate. The Radiochemical purity (RCP) of the dendrimer-amiloride complexes with Technetium-99m, achieved under ideal conditions (28 minutes of incubation, 1.4 units of reduced agent, and 17.5 mg of dendrimer-amiloride), exceeds 90%. This demonstrates the considerable potential of dendrimer-amiloride in forming complexes with Technetium-99m. The results from imaging and biodistribution tests showed that ^{99m}Tc-dendrimer–amiloride had a high level of activity (7.8 %ID/g) at the tumor site. This was due to the increased expression of sodium channel.

Conclusion: The favorable characteristics and conduct of the produced nanoprobe indicate its potential as an innovative tool for the advancement of radiopharmaceutical-based medication. Furthermore, it has the capacity to envision a broad spectrum of malignancies.

Keywords: *Amiloride, Biodistribution, Dendrimer, Spect imaging*

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INTRODUCTION

Breast cancer is a widespread and possibly lethal ailment that impacts millions of women globally [1]. Prompt identification is critical for effective therapy, rendering breast cancer imaging an essential element in combating this illness [2-4]. The significance of breast cancer imaging resides in its capacity to identify anomalies at an early phase when therapy is most efficacious. Mammography, a prominent imaging modality, can detect minute cancers or questionable alterations in breast tissue prior to their palpability [5]. Regular screenings can result in early intervention, hence greatly enhancing the likelihood of effective outcomes. Precise imaging is crucial in customizing treatment regimens. Distinct subtypes of breast cancer may

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necessitate diverse therapy approaches. Imaging plays a crucial role in assessing the scope of disease dissemination, allowing healthcare professionals to make recommendations for surgery, chemotherapy, radiation, or a combination of these treatments tailored to the specific needs of each patient [6-8].

Dendrimers, distinct macromolecules with precisely specified structures, have arisen as intriguing contenders for enhancing cancer imaging methodologies. Dendrimers are intricately structured, three-dimensional polymers with extremely small dimensions at the nanoscale [9]. Their highly regulated structure enables customized alterations, rendering them excellent contenders for diverse biomedical uses, including cancer imaging [10]. Dendrimers generally comprise a central core, branching units, and functional surface groups. Dendrimers has intrinsic characteristics that make them highly suitable for cancer imaging [11]. Their extremely small size facilitates effective penetration into tissues, while their ability to bind to several targets allows for the attachment of imaging agents and targeting ligands. In addition, dendrimers have the ability to encapsulate contrast chemicals, hence increasing the sensitivity of imaging. The process of modifying the surfaces of dendrimers with targeting molecules allows for precise identification and binding to cancer cells. This focused strategy improves the differentiation of images by specifically gathering imaging agents at the location of the tumor, reducing unwanted background signal and enhancing the precision of cancer detection [12-15].

Amiloride, a widely recognized diuretic primarily used for regulating fluid balance and blood pressure, is gaining recognition for its potential as a target drug in cancer treatment [16]. The change in emphasis arises from the distinct biological mechanisms affected by amiloride, which creates opportunities for novel strategies in cancer detection and diagnostics. Amiloride is an interesting candidate for cancer imaging since it has the capacity to block sodium-hydrogen exchangers (NHEs) [17]. These transmembrane proteins are essential for controlling the pH inside cells, a process that becomes disrupted in numerous cancer cells. Tumors frequently display an acidic microenvironment, which is a distinctive feature of cancer and a possible focus for diagnostic approaches. Scientists are investigating the potential of using amiloride as a targeted agent by creating imaging agents that are linked to amiloride [18]. These specialized medicines are specifically engineered to target and accumulate in cancer cells, exploiting the changed pH conditions found within tumors. This focused strategy has the potential to provide enhanced visual differentiation, allowing for more precise identification and characterization of malignant tissues. Amiloride has the potential to be used as a targeting agent in many imaging techniques, such as positron emission tomography (PET), magnetic resonance imaging (MRI), and fluorescence imaging. The adaptability of this technology enables a thorough investigation of its efficacy in many clinical situations, potentially leading to the development of individualized and accurate cancer imaging approaches [19].

This study presents the development of a second-generation biocompatible dendrimer-amiloride conjugate that has been radiolabeled with ^{99m}Tc. The conjugate serves as a nanoprobe for SPECT imaging. The purpose of this nanoconjugate, which contains a pegylated second generation dendrimer and amiloride, is to facilitate precise imaging of breast cancer by the use of radioisotopes. Biodistribution investigations and single-photon emission computed tomography (SPECT) scans were conducted on BALB/c mice with 4T1 tumors. These experiments provided vital information about the behavior and potential of the created radiotracer for targeted imaging in living organisms.

MATERIALS AND METHODS

Synthetic procedures

Dendrimer synthesis

In summary, a solution containing 1 mL (3.7 mmol) of polyethylene glycol (PEG) 600 from Merck, Darmstadt, Germany, was diluted in 10 mL of Dimethyl sulfoxide (DMSO) from the same source. Subsequently, a quantity of 0.75 grams (equivalent to 2 multiplied by 3.7 millimoles) of N, N'-Dicyclohexylcarbodiimide (DCC) from Merck in Darmstadt, Germany, was introduced into the solution. The reaction was prolonged for a duration of 30 min at ambient temperature with continuous stirring. Next, 0.71 grams (equivalent to 2*3.7 millimoles) of citric acid from Merck in Darmstadt, Germany, was added. The reaction was continuously agitated at ambient temperature

for a duration of 1 hour. Subsequently, 2.25 grams (equivalent to 6 times 3.7 millimoles) of DCC and 5 millilitres of DMSO were introduced. The reaction proceeded for approximately 15 minutes under the previously stated conditions. Afterwards, 2.1 grams (equivalent to 6*3.7 millimoles) of citric acid was introduced into the mixture. The reaction was then allowed to proceed for a duration of 1 week at room temperature, with continuous stirring. Subsequently, the ALGDG2 underwent two rounds of filtration. The purification process involved the utilization of a Sephadex G-50 fine column manufactured by GE Healthcare Life Sciences in the United Kingdom (Fig. 1). The fraction that was collected underwent further purification using consecutive dialysis using a dialysis bag with a cut-off of 500-1000 Da. The dialysis process was carried out against double deionized water (D.D.W) for a duration of 2 days, with a volume of 1 L. The ALGDG2 was subjected to freeze drying using the LyoTrap plus equipment from LTE Scientific Ltd in Oldham, UK. The freeze-dried ALGDG2 was then stored at a temperature of -20 °C for future research purposes.

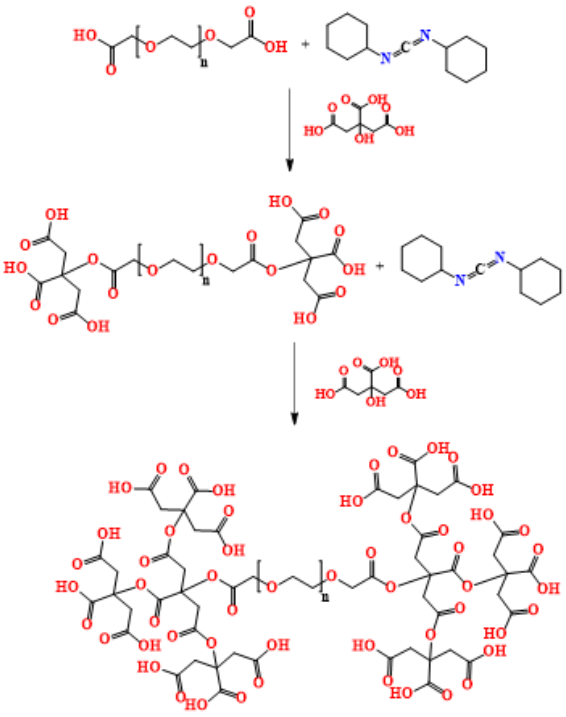

Fig. 1. Dendrimer synthesis method

Dendrimer-amiloride conjugation

In order to conjugate amiloride with the dendrimer, a solution was prepared by dissolving 20 mg of dendrimer in a mixture of 4 ml of water and dimethylformamide. To this solution, 0.02 mmol of EDC and 0.01 mmol of NHS were added. The mixture was then allowed to stand for thirty minutes. The solution's pH was subsequently modified to 9 using a buffer and then subjected to a 7-hr consumption period by adding 20 mg of amiloride. Following the completion of the reaction, the purification process was conducted (Fig. 2).

Characterization

FTIR analysis was employed to identify the functional groups present in the dendrimer by analyzing its FTIR spectra. The spectrum is

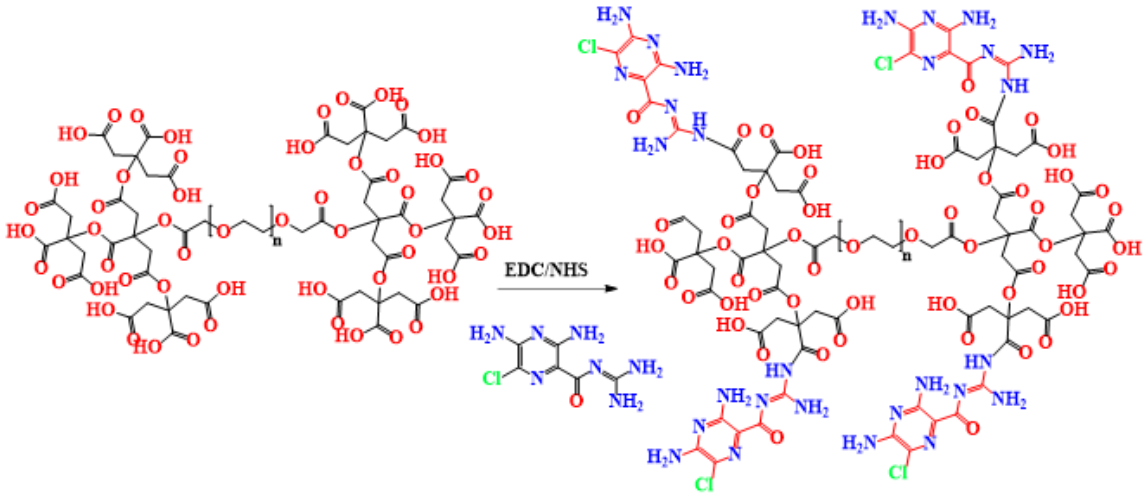

Fig. 2. Dendrimer-Amiloride Conjugation using EDC/NHS as Crosslinker

prepared by grinding a tiny quantity of lyophilized conjugated sample with potassium bromide powder. The resulting powder is then transferred into a metal mold and compressed using a hydraulic press. Subsequently, a transparent tablet is created for the purpose of evaluating the FT-IR spectrum using the designated apparatus. Ultimately, the screen displays peaks which allow for the identification of functional groups, resulting in the determination of the sample's structure.

The operator performed TEM analysis to determine the particle size once the material was delivered, prepared, and photographed in the Day Petronic laboratory, following the prescribed procedures. The software Image J was utilized to determine the dimensions of the particles.

In ideal laboratory settings, a minimal quantity of linked nanoparticles was introduced into a glass container together with 1 ml of deionized water as the solvent. In order to achieve particle dissolution and transparency, the solution underwent filtration using a 0.5-micron filter and was subjected to sonication for a duration of 10 minutes. Subsequently, it underwent analysis using a zeta sizer.

Toxicity test

This approach involves adding 100 µl of growth medium containing 30,000 cells to each well of a 96-well plate. Following a 24 hr incubation period, cells were treated with several doses of conjugate and incubated for a further 72 hr in a CO_2 incubator. Subsequently, 20 µl of MTT solution with a concentration of 5 mg/ml was added to each well and incubated in darkness for an additional 4 hr. Following the required duration, the culture medium containing MTT was cautiously extracted and 200 µl of acidified isopropanol was introduced to each well to dissolve the purple formazan. Following a 15-min period of incubation at ambient temperature, the absorbance of each well was measured using ELISA at a wavelength of 570 nm, with a reference wavelength of 690 nm. For the purpose of cell investigations, two cell lines, namely HEK-293 and MCF-7, were employed in this work.

Preparation of the kit

The kit was prepared by dissolving 10 mg of nano-conjugated with 1.8 mg of ascorbic acid in 0.9 ml of double-distilled water and adding 30 µl of Tin chloride solution. The mixture was then subjected to lyophilization for 48 hr. In order to label the kit, a specified volume of activity was introduced into the vial, ranging from 1 to 5 ml. After an adequate duration, the radiochemical purity was assessed. A central composite design (CCD) was employed for kit optimization.

Quality control for radiochemicals

This study used Whatman paper number 2 as a solid phase, two systems of 1:1 acetone/methanol as a fluid, and saline solution as a mobile phase to check the radiochemical quality. About 5 µl of the labeled kit was used to stain the strip. The stain was put 1 cm from the bottom of the strip and the strip was put into a chromatography tank. The mobile phase was moved through the strip about 10 cm and left to dry. The paper was then taken off and cut into 1-cm pieces. A gamma well counter was used to count each piece. What would have happened if saline had been used? The m99TCO_2 would have stayed at the starting point and been labeled, and it would have moved with the fluid front. If acetone/methanol had been used as the solvent, $^{99m}TCO_2$ would stay at the starting point and $^{99m}TCO_2$ would move with the front of the liquid. This made it possible to figure out the percentage of radiochemical purity using the following equation:

Purity in radiochemistry = 100 -($^{m99}TCO_2$+$^{m99}TCO_4$-)

Biodistribution study

First, a small amount of a ketamine/xylazine mixture was used to put the mouse to sleep. It was put in a cell for mice, and 0.5 ml (about 1.4 mCi) of the finished substance was injected into the tail vein of each mouse. After that, it was laid flat on its back under the machine. Imaging of various tissues was used to look at tissue uptake. To see how the body looked after the injection in terms of contrast and clarity, whole-body imaging was used. The mouse was photographed 120 min after being given a radioactive substance.. then, the right tools were used in the right place to separate the desired tissue, and a dosing calibrator was used to figure out how much activity there was. A scale found out how much each part weighed. Then, the activity of each tissue was found as a share of the total activity per gram (%ID/g) using the mean standard error.

RESULTS

FTIR analysis is a commonly employed technique

in the field of chemical and bond identification for both organic and inorganic substances. It falls within the category of spectroscopic analyses. FTIR analysis involves studying the absorption of infrared radiation by the substance being analyzed. The peaks detected at 1433 cm^{-1}, 2923 cm^{-1}, 11657 cm^{-1}, and 1450 cm^{-1} correspond to hydroxyl, -CH, -C=O, and acidic hydroxyl groups, respectively. The peaks observed at 3322 cm^{-1} and 1620 cm^{-1} in the dendrimer indicate the presence of amine functional groups and the -C=C group of the drug.

These peaks were observed in comparison to the pure spectrum of the drug in the final product's structure. Furthermore, the observed shift towards a lower wave number in the peak associated with the carbonyl group provides evidence of the drug'seffective binding to the dendrimer. Fig. 3 provides a representation of other functional groups.

The dimensions and morphology of the nanoprobe were examined using transmission electron microscopy (TEM) imaging technology, both before and after connection. This analysis is illustrated in Fig. 4. The TEM investigation yielded spherical particles ranging in size from 9.8 to 3.2 nm, with an average size of 4.38±0.67 nm.

Chemical composition analysis is crucial for identifying and characterizing unknown phases in the microstructure of materials. MAP analysis is employed for both structural analysis and elemental analysis of a material (Fig. 5). By integrating it into electron microscopes, one may examine the backscattered rays from the sample and ascertain the distinctive X-ray of each element, utilizing the resolution and capability of the instrument. He conducted several examinations, including magnification, qualitative, and quantitative analysis, on a diverse array of samples. The study revealed the presence of oxygen, chlorine, carbon, and nitrogen components, confirming the successful synthesis of the final product. The obtained results demonstrated satisfactory concordance with the MAP analysis. Moreover, the analysis of the failures detected in the MASS spectrum revealed the effective linkage of the medication to the dendrimer.

The specificity of mass spectra ensures that the findings acquired from qualitative and quantitative examination are highly reliable. Each species has its unique mass spectrum, which serves as the identification of that particular item. Mass spectrometers exhibit higher sensitivity and specificity for most substances compared to alternative liquid chromatography detectors. The MASS analysis data obtained from this molecule confirm the effective synthesis and the existence of the medicine in this structure. Here are a few of the breakdowns that are currently accessible.

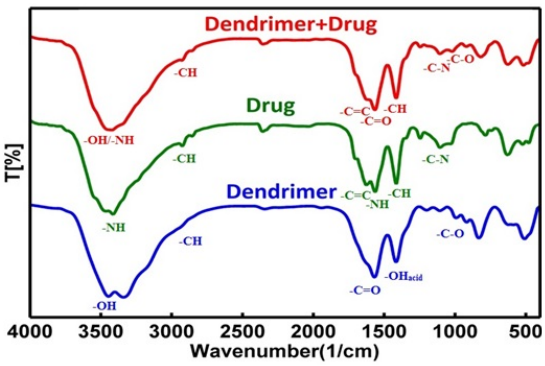

Fig. 4. (a) TEM image and (b) Size distribution of dendrimer

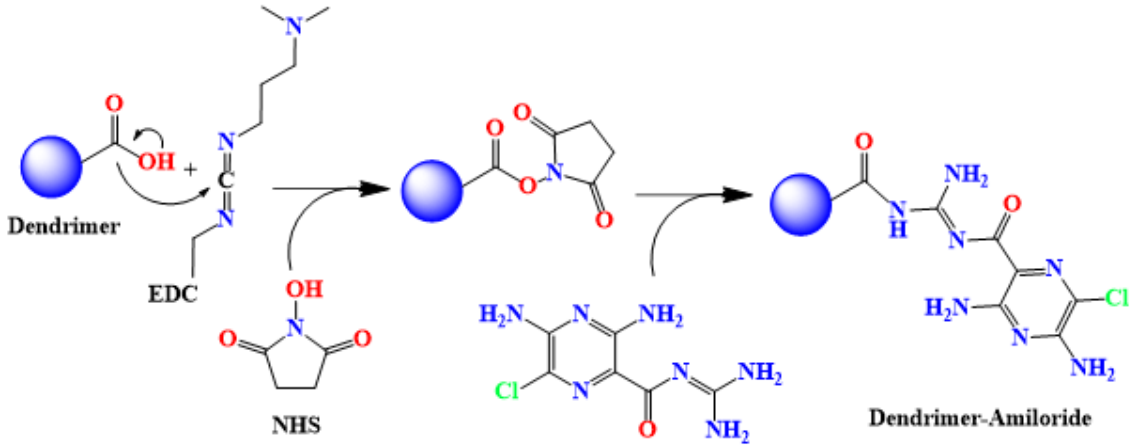

Fig. 3. FTIR spectrum of dedndrimer(blue), drug (green) and drug-dendrimer conjugate (red)

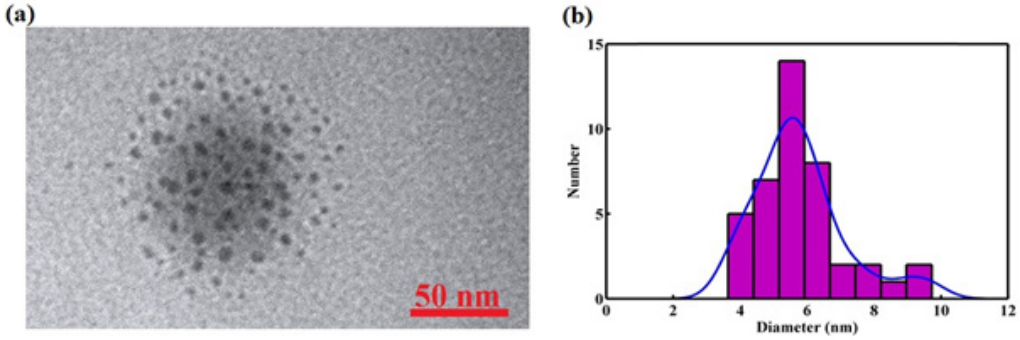

Fig. 5. Elemental Map analysis of final product

The bell-shaped picture signifies the presence of polyethylene glycol in the synthesized compound (Fig. 6).

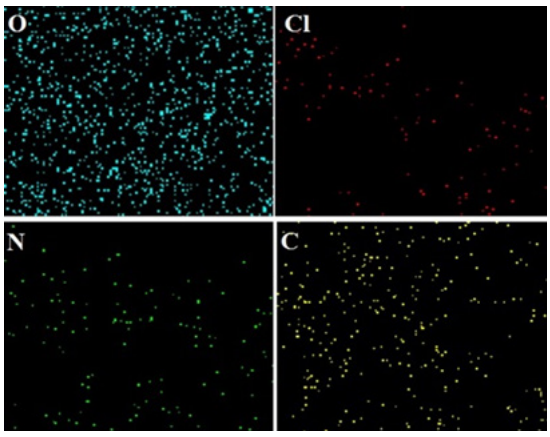

Fig. 6. Mass spectrum of drug-dendrimer conjugate

To optimize the labelling efficiency, an investigation was conducted to analyze the impact of various aspects. The response level method based on the Bencken box was employed to analyze all the variables. In order to achieve this objective, the variables of labelling time, ligand quantity, and quantum dot quantity, which have an impact on labelling efficiency, were investigated at three different levels (Table 1). The design involved selecting a ligand quantity ranging from 10 to 20 mg, a reducing agent quantity ranging from 1 to 4 mg, and a time duration ranging from 5 to 35 min. This approach enables the responses to be represented by fitting a mathematical equation. There were a total of 20 tests conducted, and the details of each test along with the outcomes are shown in Table 2. The modelling process involved utilizing three parameters: two product terms to account for the interactions between the key parameters, and quadratic terms for each of the main parameters to capture the non-linear connection with the response variable. To conduct a more in-depth investigation, an analysis of variance was carried out using a quadratic mathematical model. The findings of the analysis of variance on the modelling are shown in table [3]. P-values less than 0.05 in the modelling procedure indicate the significance of the effect of each sentence employed. The findings indicated that, with the exception of time, time square, and probe square, the remaining variables were not statistically significant. The positive sign indicates a direct relationship between the parameter and the response, while the negative sign indicates an inverse association. The size of the coefficient reflects the strength of the parameter's effect. In this study, increasing each of these circumstances leads to an increase in labelling. Below is the figure depicting the border of the two-dimensional vision.

In the time-reduction factor interaction diagram, the labelling efficiency has exhibited a positive correlation with the duration, however no correlation between the reducing factor and the labelling efficiency was detected in this interaction. During the time-probe interaction, the efficacy of labelling was found when the sample amount employed ranged from 15 to 20 milligrams. The

Table 1. Coded values design for optimization of each parameter

Factor	Name	Unit	-1	0	1
A	Time	Min	5	20	35
B	Reduced agent	Mg	1	2.5	4
C	Probe	Mg	10	15	20

Table 2. Radio chemical purity results obtained for experimental design

StdOrder	RunOrder	PtType	A	B	C	Resp
7	1	1	-1.00	-1.00	-1.00	59
16	2	0	-1.00	-1.00	1.00	61
9	3	-1	-1.00	1.00	-1.00	64
20	4	0	-1.00	1.00	1.00	65
4	5	1	1.00	-1.00	-1.00	84
5	6	1	1.00	-1.00	1.00	89
8	7	1	1.00	1.00	-1.00	83
1	8	1	1.00	1.00	1.00	87
14	9	-1	-1.68	0.00	0.00	43
3	10	1	1.68	0.00	0.00	90
11	11	-1	0.00	-1.68	0.00	73
18	12	0	0.00	1.68	0.00	80
12	13	-1	0.00	0.00	-1.68	73
15	14	0	0.00	0.00	1.68	74
13	15	-1	0.00	0.00	0.00	79
10	16	-1	0.00	0.00	0.00	82
19	17	0	0.00	0.00	0.00	81
2	18	1	0.00	0.00	0.00	82
17	19	0	0.00	0.00	0.00	85
6	20	1	0.00	0.00	0.00	80

Table 3. ANOVA results for experimental design study

Term	Coef	SE Coef	T-Value	P-Value
Constant	81.42	1.06	76.90	0.000
A	12.676	0.703	18.03	0.000
B	1.302	0.703	1.85	0.094
C	1.003	0.703	1.43	0.184
A*A	-4.802	0.685	-7.01	0.000
B*B	-1.259	0.685	-1.84	0.096
C*C	-2.322	0.685	-3.39	0.007
A*B	-1.500	0.918	-1.63	0.133
A*C	0.750	0.918	0.82	0.433
B*C	-0.250	0.918	-0.27	0.791

improvement in labelling efficiency was noticed when the operation was conducted for an extended duration, specifically around thirty-five minutes. The sample-reducing agent interaction showed the highest labelling efficiency within the range of 17.5-12.5 mg for the sample and 4-1.75 mg for the reducing agent (Fig. 7).

Cell culture plays a crucial role in the advancement of novel pharmaceuticals. Various cellular assays require the examination of distinct molecules, extracted natural compounds, or separated useful drugs to assess their toxicity. In order to comprehend the impact of a substance on cells, it is imperative that we possess the ability to ascertain the quantity of viable cells within the cell culture. MTT assay is a highly prevalent test

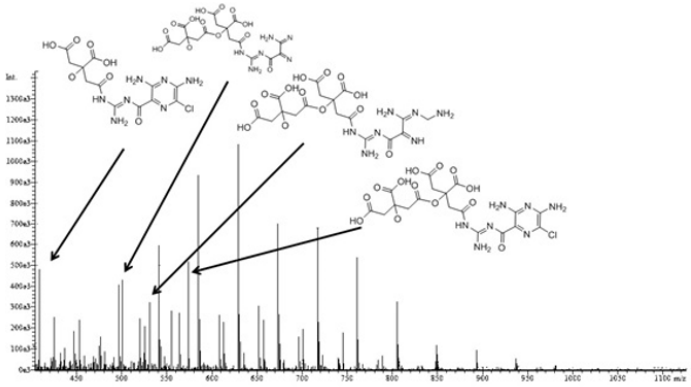

Fig. 7. Contour plot of (a) time-reduced agent, (b) time-probe and (c) reduced agent-probe obtained from experimental design

in cellular laboratories. This test assesses the cell's viability by evaluating its metabolic activity, which is indicative of the cell's ability to survive. The investigation revealed that neither substance exhibited noteworthy toxicity up to a concentration of 50 µg/ml. However, when the concentration increased to 500 µg/ml, around 40% of the cells' viability was declined. It is important to mention that the drug's attachment to the dendrimer did not exhibit any alteration in activity (Fig. 8).

The imaging results revealed the spatial arrangement within the deceased organs. The biodistribution investigation revealed that the kidney, liver, and spleen exhibited the highest levels. Based on the drug's hepatic metabolism, the accumulation in the liver suggests that there is no alteration in the drug's pharmacokinetics. On the other hand, the accumulation in the tumor reveals the compound's affinity for the tumor, making it a potential marker. Furthermore, the presence of the molecule in the liver and bladder suggests that it has a strong affinity for water and is eliminated from the body through the kidneys (Fig. 9).

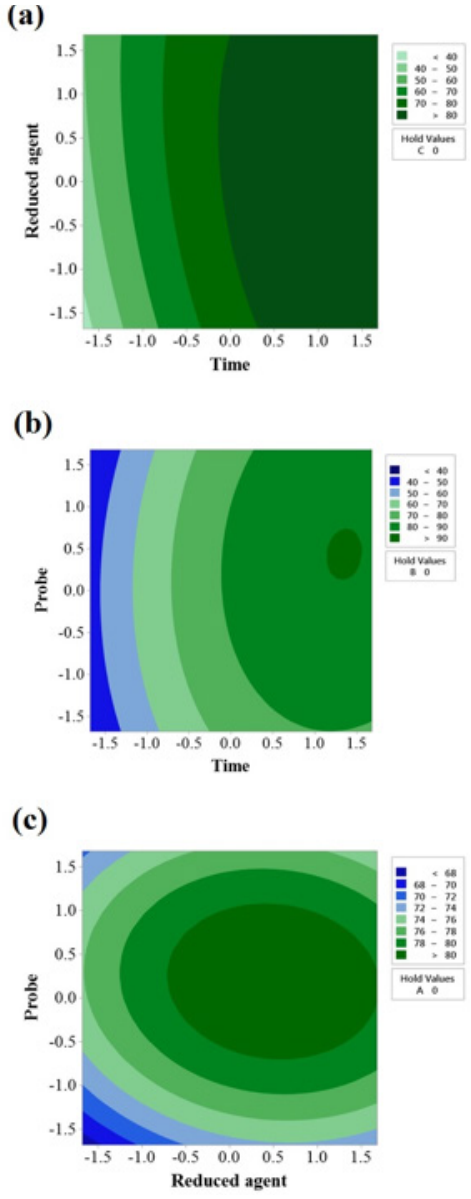

Fig. 8. Investigation of toxicity of amiloride (blue) and denrimer-amiloride (red) on HEK-293 cell line

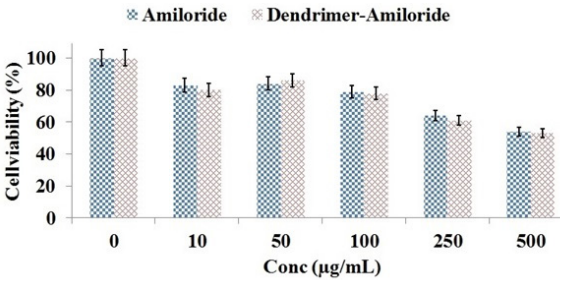

Fig. 9. (a) SPECT imaging and (b) Biodistribution of ^{99m}Tc-dendrimer-amiloride on cancerous mice

DISCUSSION

Medicines as targeting agents are a unique and intriguing method with extensive implications, notably for imaging technologies. These platforms improve therapeutic effects in specific tissues, cellular absorption, side effects, and diagnostic precision and sensitivity. Multiple studies have shown drug-coated nanoparticles to be effective nuclear imaging agents. This novel technique could expand oncology and other therapeutic and diagnostic uses. Van Dijl et al. employ vancomycin tagged with a fluorescent dye (vanco-800CW) to diagnosis Gram-positive bacteria illnesses. Vanco-800CW being tested for real-time in vivo imaging of bacterial infections in mice myositis and human post-mortem implant models. In our mouse myositis model, vanco-800CW accurately detected Gram-positive bacterial infections (20). In addition, it can distinguish bacterial infections from sterile inflammation in living organisms and biomaterial infections in the lower leg of a deceased human. Nanoparticle conjugation, radiolabeling, and a targeted ligand can improve cancer imaging accuracy and specificity. Nanoparticle scientists are interested in dendrimers. Dendrimers have precise size, structure, and surface properties. These traits enable the exact synthesis of chemicals that specifically target cancer cells, distinguish malignant from healthy tissue, and directly deliver

imaging agents to tumors. High accuracy is needed to detect cancer early, provide accurate diagnoses, and evaluate patient treatment. Dendrimers can be tailored with antibodies, peptides, or small molecules that bind to cancer cells, making them useful in cancer imaging. A tailored technique ensures that dendrimer-carried imaging agents aggregate mostly in tumor tissues. Thus, non-specific background signaling is reduced and image sharpness improved. This allows therapists to receive more exact and detailed visual portrayals of tumors, enabling more accurate and precise therapies(21). SPECT imaging of malignant tissue by Qureshi et al. used dendrimer-glutamine architecture. Without a chelator, the synthesized structure efficiently complexed with technetium (22). Li et al. used SPECT/CT to view cancer tissues using a modified second-generation gold dendrimer structure functionalized with folic acid (23).

This study is significant as it emphasizes the advancement of second-generation dendrimers, their successful combination with amiloride, and their subsequent labeling with ^{99m}Tc for SPECT imaging in mice with 4T1 tumors. The connection between the metal and the dendrimer is formed through the exchange of electrons from the oxygens of citric acid to the metal. The synthesis of polyethylene glycol-citrate dendrimer, as described in the articles, requires a series of intricate procedures, which can be quite time-consuming and expensive. In addition, the method involves the use of potentially dangerous chemicals like dichloromethane and pyridine. Thus, this study has effectively reduced both response time and the duration needed to obtain the product. In addition, this method utilizes chemicals with significantly reduced levels of toxicity. This innovative method utilized activators, such as DCC, which significantly reduced toxicity, thereby minimizing the reliance on hazardous substances like dichloromethane and pyridine. Amiloride was attached to the surface functional groups of the dendrimer using the NHS/EDC crosslinker. The crosslinker does not have any spacers (24). This crosslinker enables the formation of a covalent bond between the carboxyl groups of the dendrimer and the amine of amiloride, just like a pharmacologist would do. Here is an outline of the synthesis mechanism. Amiloride exploits these differences by selectively binding to sodium channels that are excessively produced in cancer cells (25). With the precision of a skilled professional, this approach ensures that imaging agents are delivered directly to cancerous tissues, while sparing healthy cells from harm. As a result, the likelihood of experiencing negative side effects is significantly reduced. After conducting a statistical analysis of the data from the MTT experiment, it was found that both amiloride and the produced nano conjugate did not show any significant toxicity on the HEK-293 cells, which were used as control cells, at any dosage during a 48-hour exposure period. With its low toxicity and excellent safety profile, amiloride offers a safer option compared to traditional imaging agents, thereby reducing the potential risks to patients. An alternative method that does not require the use of chelators can be used to conveniently label nanoparticles with radioactive materials. This technology simplifies the process of attaching a chelator, preserving the nanoparticles' surface charge, size, and ability to disperse and migrate within a live organism. Moreover, the straightforward attachment of radiometals to nanoparticles via surface interactions that are uncomplicated, effective, and cost-effective enables the transition from preclinical testing to clinical application. Through this study, the use of denrimer has proven to be highly effective in enhancing the precision and excellence of imaging techniques. Similar to a pharmacologist, the ^{99m}Tc-dendrimer-amiloride has numerous advantageous features. These include its simple synthesis process, safety, easy availability, and cost-effectiveness of ^{99m}Tc. Furthermore, it achieves a remarkable level of radiochemical purity through the use of chelator-based labeling. It demonstrates excellent stability under controlled conditions, possesses impressive imaging capabilities, and exhibits a favorable distribution pattern within the body. However, additional thorough investigations are necessary to fully understand and describe all the features of this radiotracer for its potential use in clinical settings in the near future.

CONCLUSION

This study presents a new type of imaging probes that use second generation dendrimer. These probes were combined with amiloride and then labeled with ^{99m}Tc using a simple and effective method. The investigation involved the use of SPECT imaging and *in vivo* biodistribution studies performed on mouse models. The radiotracer accumulates in the tumor due to the increased

expression of sodium channels on the tumor. With the use of this radiotracer, renal clearance becomes much safer, which is essential for its clinical applications. Developing and synthesizing these contrast agents can greatly enhance our knowledge of how drugs function in the body as targeting agents. This, in turn, offers valuable insights into the creation of targeted contrast agents.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Ye F, Dewanjee S, Li Y, Jha NK, Chen ZS, Kumar A, Vishakha, Behl T, Jha SK, Tang H. Advancements in clinical aspects of targeted therapy and immunotherapy in breast cancer. Molecular cancer. 2023;22(1):105.
2. Ashrafizadeh M, Zarrabi A, Bigham A, Taheriazam A, Saghari Y, Mirzaei S, Hashemi M, Hushmandi K, Karimi-Maleh H, Nazarzadeh Zare E, Sharifi E. (Nano) platforms in breast cancer therapy: Drug/gene delivery, advanced nanocarriers and immunotherapy. Med Res Rev. 2023;43(6):2115-2176.
3. Ye F, Dewanjee S, Li Y, Jha NK, Chen ZS, Kumar A, Vishakha, Behl T, Jha SK, Tang H. Advancements in clinical aspects of targeted therapy and immunotherapy in breast cancer. Molecular cancer. 2023;22(1):105.
4. Nolan E, Lindeman GJ, Visvader JE. Deciphering breast cancer: From biology to the clinic. Cell. 2023; 186(8):1708-1728.
5. Munnink TO, Nagengast WB, Brouwers AH, Schröder CP, Hospers GA, Lub-de Hooge MN, Van der Wall E, Van Diest PJ, De Vries EG. Molecular imaging of breast cancer. Breast. 2009;18:566-573.
6. Iranmakani S, Mortezazadeh T, Sajadian F, Ghaziani MF, Ghafari A, Khezerloo D, Musa AE. A review of various modalities in breast imaging: technical aspects and clinical outcomes. Egyptian Journal of Radiology and Nuclear Medicine. 2020;51(1):1-22.
7. Na SP, Houserkovaa D. The role of various modalities in breast imaging. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2007;151(2):209-218.
8. Sree SV, Ng EY, Acharya RU, Faust O. Breast imaging: A survey. World J Clin Oncol. 2011; 2(4): 171–178.
9. Patel HN, Patel PM. Dendrimer applications—A review. Int J Pharm Bio Sci. 2013;4(2):454-643.
10. Qiao Z, Shi X. Dendrimer-based molecular imaging contrast agents. Progress in polymer science. 2015;44:1-27.
11. Li H, Zha S, Li H, Liu H, Wong KL, All AH. Polymeric dendrimers as nanocarrier vectors for neurotheranostics. Small. 2022;18(45):2203629.
12. Ambekar RS, Choudhary M, Kandasubramanian B. Recent advances in dendrimer-based nanoplatform for cancer treatment: A review. European polymer journal. 2020;126:109546.
13. Wolinsky JB, Grinstaff MW. Therapeutic and diagnostic applications of dendrimers for cancer treatment. Adv Drug Deliv Re. 2008;60(9):1037-1055.
14. Cai X, Hu J, Xiao J, Cheng Y. Dendrimer and cancer: A patent review (2006–present). Expert Opin Ther Pat. 2013;23(4):515-529.
15. Saluja V, Mishra Y, Mishra V, Giri N, Nayak P. Dendrimers based cancer nanotheranostics: An overview. International Journal of Pharmaceutics. 2021;600:120485.
16. Rowson-Hodel AR, Berg AL, Wald JH, Hatakeyama J, VanderVorst K, Curiel DA, Leon LJ, Sweeney C, Carraway III KL. Hexamethylene amiloride engages a novel reactive oxygen species-and lysosome-dependent programmed necrotic mechanism to selectively target breast cancer cells. Cancer letters. 2016;375(1):62-72.
17. Amith SR, Fliegel L. Na+/H+ exchanger-mediated hydrogen ion extrusion as a carcinogenic signal in triple-negative breast cancer etiopathogenesis and prospects for its inhibition in therapeutics. InSeminars in cancer biology 2017; 43:35-41.
18. Cardone RA, Alfarouk KO, Elliott RL, Alqahtani SS, Ahmed SB, Aljarbou AN, Greco MR, Cannone S, Reshkin SJ. The role of sodium hydrogen exchanger 1 in dysregulation of proton dynamics and reprogramming of cancer metabolism as a sequela. Int J Mol Sci. 2019;20(15):3694.
19. Poku LO, Phil M, Cheng Y, Wang K, Sun X. 23Na-MRI as a Noninvasive Biomarker for Cancer Diagnosis and Prognosis. J Magn Reson Imaging. 2021;53(4):995-1014.
20. Van Oosten M, Schäfer T, Gazendam JA, Ohlsen K, Tsompanidou E, De Goffau MC, Harmsen HJ, Crane LM, Lim E, Francis KP, Cheung L. Real-time *in vivo* imaging of invasive-and biomaterial-associated bacterial infections using fluorescently labelled vancomycin. Nature communications. 2013;4(1):2584.
21. Saluja V, Mishra Y, Mishra V, Giri N, Nayak P. Dendrimers based cancer nanotheranostics: An overview. International Journal of Pharmaceutics. 2021;600:120485.
22. Ghoreishi SM, Khalaj A, Sabzevari O, Badrzadeh L, Mohammadzadeh P, Mousavi Motlagh SS, Bitarafan-Rajabi A, Shafiee Ardestani M. Technetium-99m chelator-free radiolabeling of specific glutamine tumor imaging nanoprobe: *in vitro* and *in vivo* evaluations. Int J Nanomed. 2018:4671-6483.
23. Li X, Xiong Z, Xu X, Luo Y, Peng C, Shen M, Shi X. ^{99m}Tc-labeled multifunctional low-generation dendrimer-entrapped gold nanoparticles for targeted SPECT/CT dual-mode imaging of tumors. ACS Appl Mater Interfaces. 2016;8(31):19883-19891.
24. Wong SS, Jameson DM, Wong SS. Chemistry of protein and nucleic acid cross-linking and conjugation. Boca Raton, FL, USA: CRC Press; 2012.
25. Fraser SP, Diss JK, Chioni AM, Mycielska ME, Pan H, Yamaci RF, et al. Chemistry of protein and nucleic acid cross-linking and conjugation. Clin Cancer Res. 2005;11(15):5381-5389.