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Research article

## RADIOLABELING AND RADIATION DOSIMETRY OF $^{99m}\text{Tc}$ -ZOLEDRONIC ACID ( $^{99m}\text{Tc}$ -ZA) FOR BONE SCINTIGRAPHY

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### ABSTRACT

**Introduction and Objective:** Search of a target specific radiopharmaceutical is always on and the development of radiolabeling methods for a target specific bone scintigraphy agent is need of hour for timely management of cancer. Zoledronic acid belongs to a class of drugs known as bis phosphonates. Technitium-99m has good gamma imaging characteristics so it can be good osteoscintigraphy radio pharmaceutical. Analysis the radiation dosimeter is also very important for any radiopharmaceutical. **Material and Methods:** High grade reagent and radiopharmacy laboratory of Nuclear Medicine department of Bhagwan Mahaveer Cancer Hospital & Research Centre (BMCHRC), Jaipur used. We have procured all the radioisotopes and other chemicals. Radiolabeling has been done as per standard radiochemical reactions. Quality control of radio labeled  $^{99m}\text{Tc}$ -ZA has been done by chromatographic techniques. Radiation Dosimetry has been done as per guidelines of the radiation survey and monitoring of MIRD. **Results/Discussion:** We have successful radiolabeled the Zoledronic acid with  $^{99m}\text{Tc}$ . We have detected 98.55 % radiolableing efficiency of ZA with  $^{99m}\text{Tc}$ . The radiopharmaceutical can be used for clinical trial and application after further testing. We have also observed good radiation dosimetry parameters for diagnostic use of this radiopharmaceutical. The Radiopharmaceutical can be good nuclear medicine radiopharmaceutical for the osteoscintigraphy. **Conclusion:** The discussed radiolabeling techniques and methods for technetium labeled Zoledronic Acid has shown good radiolabeling efficiency and good radiation dosimetry characteristics that are very encouraging. It can be used for bone scintigraphy further testing.

**Keywords:** Zoledronic Acid (ZA), Acid, Osteoscintigraphy, SPECT, Bisphosponate, ITLC, Dosimetry.

### INTRODUCTION

There are many osteoscintigraphy radiopharmaceuticals available and search for the best is always on. It is expected from osteoscintigraphy radiopharmaceuticals to have high target specific radiopharmaceutical for better nuclear medicine imaging results. The Zoledronic acid belongs to a class of drugs known as bisphosphonates. Bisphosphonates are used to treat bone diseases such as osteoporosis and cancer-induced bone pain and fracture. It is an anti hyper calcemic drug and lowers high blood calcium levels by reducing the amount of calcium released from your bones into your blood. The drug used to treat hypercalcemic blood level induced by bone malignancy.

Bisphosphonates show negligible biotransformation. The radiolabeling of Zoledronic acid with Technetium 99m can has a potential radiopharmaceutical for bone imaging because of its higher affinity to bone tissue. It can also provide efficient bone gamma imaging parameters with for bone imaging.

As a result of clinical high efficacy, safety and cost effectiveness of  $^{99m}\text{Tc}$ - Zoledronic Acid (ZA) were demonstrated for bone scanning in patients with pathological process in bones. The drug provided visualization of both osteoclastic and osteolytic metastases, enabled to carry out differential diagnostics of bone lesions, facilitate decision of treatment

tactics and control outcomes thereof. Bone scanning utilizing  $^{99m}\text{Tc}$ -ZA allow to reduce examination time by 67% while obtain images of higher quality. The development of radiolabeling methods for a target specific bone scintigraphy agent is need of hour for timely management of cancer. Previous animal and clinical studies has shown that the ZA labeled with  $^{99m}\text{Tc}$  has high degree of affinity to bone tissues. In this study we have developed the radiolabing methods for  $^{99m}\text{Tc}$ -ZA and conducted quality control for the newly developed  $^{99m}\text{Tc}$ -ZA.

## MATERIAL AND METHODOLOGY

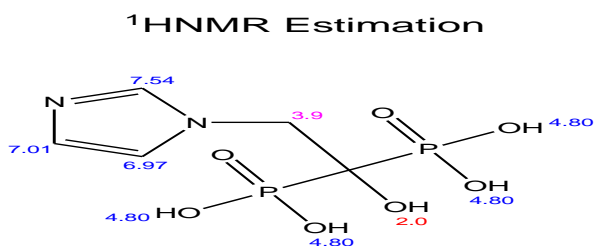
### Chemical

$^{99m}\text{TcO}^{4-}$  is procured in the form of  $^{99}\text{Mo}/^{99m}\text{Tc}$  Generator from Paras Isotope, Iran. Zoledronic Acid kit was a kind of gift from Sanlar Imex, Mumbai. Other chemicals of high and analytical grade were used from hospital radiopharmacy laboratory without further purification. 0.22 micron syringe filter imported from Millex Millore, Merck, Ireland. Radio Chemical Purity testing kits (Paper and ITLC) kits procured from from BRIT, Mumbai. Other equipments, instruments and consumables used form radiopharmacy laboratory. Equipments: Gamma Counter, SPECT Gamma camera, Symbia, Siemens, Germany, for data acquisition and analysis. Other computer and software also used for the analysis of the data.

### Zoledronic Acid Kit

ZA Molecular Formula:  $\text{C}_5\text{H}_{10}\text{N}_2\text{O}_7\text{P}_2$

Figure 1. Molecular Structure of Zoledronic Acid



### Pharmaceutical kit preparation for the Zoledronic Acid:

The formulated kit contained a white or nearly white powder in the form of cakes or individual units, or powder. In Solution it's a colorless transparent liquid. One kit contained 1.5 mg quantity of ZA along with 0.33mg of Tin dichloride anhydrous ( $\text{SnCl}_2$ ).

### Inclusion and Exclusion criteria

The cases amongst the patients referred for the bone scintigraphy were randomly selected. No pregnant females and children below 18 years of age were

included. Only those cases were selected who did not have any kind of intervention including chemotherapy, radiotherapy or surgery for their condition.

Patients were advised to take plenty of fluids before coming for the study.

### Administration of Radiopharmaceutical

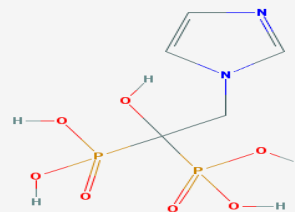
Radiopharmaceutical was administered intravenously as per the pre decided dose with the help of IV cannula.

$^{99m}\text{TcO}^{4-}$  is procured in the form of  $^{99}\text{Mo}/^{99m}\text{Tc}$  Generator from Paras Isotope, Iran. Chemical: Zoledronic Acid a kind of gift from Sanlar Imex, Mumbai. Other chemicals  $\text{SnCl}_2$  and other used from hospital radiopharmacy lab. If not otherwise stated, chemicals, materials and solvents were of pharmaceutical grade for kit preparation and reagent grade for other experiments and were used without further purification. 0.22 micron syringe filter imported from Millex Millore, Merck, Ireland and used for the filtration.

Radio Chemical Purity testing kit: Paper and ITLC Chromatograophy kits purchased from BRIT Mumbai and other vendors. Other equipments, instruments and consumables used form radiopharmacy lab.

Equipments: Gamma Counter, Dual Head SPECT System, Symbia of Siemens, Germany, is used for counting the strips. Other computer and software used for the analysis of the data.

Figure 2. Molecular Structure of Zoledronic Acid



ZA Molecular Formula:  $\text{C}_5\text{H}_{10}\text{N}_2\text{O}_7\text{P}_2$

The formulated kit contained a white or nearly white powder in the form of cakes or individual units, or powder. In Solution it's a colorless transparent liquid. One kit contained 1.5 mg quantity of ZA along with 0.33mg of Tin dichloride anhydrous ( $\text{SnCl}_2$ ).

## EXPERIMENT

Radiolabing Methods for Zoledronic Acid: Following steps has been used for the preparation of Zoledronic Acid (ZA) kit for the  $^{99m}\text{Tc}$ -Radiolableing

1. 5 ml of the eluate from Technetium- $^{99m}$  generator with a volume activity of 185-740 MBq/ml aseptically

injected with a syringe into the vial with the lyophilisate, the needle piercing the rubber stopper.

2. If necessary, conduct pre-dilution of the eluate with isotonic sodium chloride solution up to the required value of volumetric activity.
3. Shaking the vial contents stirred until complete dissolution of the freeze-dried.
4. The preparation is ready to use within 20 minutes after preparation.
5. The finished product, prepared on the basis of lymphilisate contained in one bottle can be used for the study, 5 patients.
- 6.

**Figure 3. Zoledronic Acid formulated kit and radiolabelling of Zoledronic Acid with 99mTc**



Patient injected radio pharmaceutical after testing the radiolabelling efficacy. IV given the drug in three category. 1.5 mci, 10 mci and 15 mci, the imaging time also in three different internal 1 hour 2 hour and 3 hours. For optimization of the radiopharmaceutical dose and imaging time.

An open comparative study was performed at the Bhagwan Mahaveer Cancer Hospital & Research Centre, Jaipur to examine sensibility and safety of technetium-labeled ZA in patients with prostate cancer in case of skeletal lesions detected by bone scanning. Frequency of coincidence and discrepancy in bone scanning results when utilizing 99mTc-ZA was evaluated. Novel drug safety was assessed by virtue of analysis of undesirable events and side effects frequency. Obtained results show equal diagnostic value of assessed drug 99mTc-ZA,

99mTc (1 h after administration) and Technifor, 99mTc (3 h after administration). No significant difference in KDA minimal values  $1,48 \pm 0,29$  vs  $1,41 \pm 0,52$  ( $p = 0,668$ ), and KDA maximal values  $2,22 \pm 1,19$  vs  $1,88 \pm 0,82$  ( $p = 0,381$ ) was observed. As a result of clinical study, high efficacy, safety and cost-effectiveness of Rezoscan, 99mTc were demonstrated for bone scanning in patients with pathological process in bones. The drug provided visualization of both osteoclastic and osteolytic metastases, enabled to carry out differential diagnostics of bone lesions, facilitate decision of treatment tactics and control outcomes thereof. Bone scanning utilizing 99mTc-ZA, 99mTc allow to reduce examination time by 67% while obtain images of higher quality. The novel drug may be recommended for wide application in clinical practice in medical institutions of the Russian Federation.

**Kit Formulation for 99mTc-ZA**

Valium for drug of a solution for the on / in a white or nearly white, in the form of cakes or individual units, or powder; prepared solution - a colorless transparent liquid.

Chemical	1 fl.
Zoledronic acid	1.5 mg

Excipients: tin dichloride anhydrous - 0.33 mg, 1 ml of final solution contained. Active substances.

Technetium 99m	185-740 MBq
zoledronic acid used	300 mcg

**Description of the active ingredients' preparation 99mTc-ZA**

Pharmacologic effect Diagnostic Radiopharmaceutical drug. Zoledronic acid is labeled with <sup>99m</sup>Tc, it has high affinity for bone. Main the freeze-dried substance - bisphosphonate - zoledronic acid, which has the maximum affinity for areas of increased metabolism and accelerated resorption of bone.

To identify the centers of pathological resorption and increased metabolism in areas of bone in various pathological processes in the skeleton:

For the detection and identification of lytic, mixed and blastic metastases in the skeleton in malignant tumors of various origins and prevalence;

Osteomyelitis, osteoarticular tuberculosis, osteoporosis, various bone and joint degenerative processes, including arthritis and arthritis of various origins;

To select the specific treatment of bone lesions drug zoledronic acid, and monitoring the effectiveness of treatment.

**Dosage**

The drug is injected in / in. Dosage is carried out in accordance with the objectives of the study and the nature of the methods and the technical specifications of the equipment used. Typically, the dose administered was 5 MBq per 1 kg of body weight.

**Methodology of the survey**

The study is carried out using a gamma camera by scintigraphy of the whole body in the front and rear projections 1-2 hours after administration of the drug with the obligatory prior emptying of the bladder. Interpretation of results of research carried out by two independent nuclear medicine physicians assessing the distribution of the  $^{99m}\text{Tc}$ -ZA in the skeleton. Areas of pathological changes in bone tissue characterized by pockets of drug hyperfixation of  $^{99m}\text{Tc}$ -ZA.

The study is carried out using a gamma camera by scintigraphy of the whole body in the front and rear projections 1-2 hours after administration of the drug with the obligatory prior emptying of the bladder.

Interpretation of results of research carried out by assessing the distribution of the drug in the skeleton. Areas of pathological changes in bone tissue characterized by foci hyperfixation  $^{99m}\text{Tc}$ -ZA preparation  $^{99m}\text{Tc}$  of Tc.

**Radiation Dosimetry of  $^{99m}\text{Tc}$ -ZA**

The radiation dose to the patient body using a drug  $^{99m}\text{Tc}$ -ZA calculated by MIRD human model in Organ: Radiation Dose (rad/MBq).

**Exclusion and Inclusive Criteria:**

Inclusive Criteria: All patients referred for the bone scintigraphy study.

Exclusion: Child and adolescence to 18 years, pregnancy, hypersensitivity to the drug or its components.

**Terms and Conditions of storage**

Lyophilisate should be stored in a dry, dark place at a temperature of 2 ° to 10 ° C. The drug should be stored in accordance with the "Basic sanitary rules of radiation safety" (OSPORB-99). Shelf life of the lyophilisate - 1 year, finished product - 5 hours from the preparation time.

**Drug interactions**

In the dosages interactions with other drugs have been observed. Instruction for medical use of the drug.

Content

- Indications, Pharmacokinetics, Use during pregnancy, Contraindications, Side effects, Dosing and Administration, Overdose, Interactions with other drugs, while taking precautions, Storage conditions, Shelf life

**Indications**

To identify the centers of pathological resorption and increased metabolism in areas of bone in various pathological processes in the skeleton:

For the detection and identification of lytic, mixed and blastic metastases in the skeleton in malignant tumors of various origins and prevalence;

Osteomyelitis, osteoarticular tuberculosis, osteoporosis, various bone and joint degenerative processes, including arthritis and arthritis of various origins;

To select the specific treatment of bone lesions drug zoledronic acid, and monitoring the effectiveness of treatment.

Pharmacokinetics

**Distribution**

Pharmacokinetic studies of ZA labeled with  $^{99m}\text{Tc}$ , showed that its distribution is characterized by severe bone-seeking on the background of the high rate of excretion of organs, tissues and whole body. Cmax of the drug in major organs and tissues including kidney, observed 10 minutes after administration. The bladder Cmax observed after 1 h after injection. Maximum drug accumulation in the skeleton (40% of the injected activity) is observed after 1 to 2 hours after administration. High accumulation of the drug in the skeleton is preserved up to 8-12 hours of observation.

Zoledronic Acid is labeled with  $^{99m}\text{Tc}$ , it has a high removal rate. The level of activity in the blood after administration of not more than 1% and 5.4 min already reduced to trace values. 1 hour after administration to about 20% of the drug excreted in the urine, there is a significant decrease in the level of the isotope in the kidneys, liver, skeletal muscle and whole body.

**Contraindications**

Child and adolescence to 18 years; Pregnancy; Lactation (breastfeeding); Hypersensitivity to the drug or its components. Side effects Perhaps: allergic reactions.

**Dosing and Administration**

The drug is injected in / in. Dosage is carried out in accordance with the objectives of the study and the nature of the methods and the technical specifications of the equipment used. Generally we have administered was 5 MBq per 1 kg of body weight.

Due to the possibility of clinically significant impairment of renal function up to renal failure, single dose should not exceed 4 mg and infusion duration should not be less than 15 minutes.

Bisphosphonates, including ZA may have a nephro toxic effect manifesting deterioration of renal function, and possibly kidney failure. In clinical studies, the risk of renal impairment (defined as an increase in serum creatinine) was significantly higher in patients who

received an infusion over 5 min, compared with patients who was administered the same dose for 15 min. Furthermore, the risk of deterioration of the renal function and renal failure was significantly higher in the group of patients treated with a dose of 8 mg, even if the duration of infusion was 15 min. While the risk is reduced by a dose of 4 mg for 15 minutes, the deterioration of renal function is likely. Risk factors for these disorders are initially elevated levels of serum creatinine and repeated cycles of bisphosphonate treatment.

Patients receiving zoledronic acid, is necessary to determine the level of serum creatinine prior to each dose. When renal function deterioration in patients with bone metastases need to cancel the introduction of the next dose. If any signs of deterioration in renal function in

patients with SRC must be a thorough examination to decide on the prevalence of the potential benefits of the use of zoledronic acid over the possible risk.

After initiation of therapy requires careful monitoring of the level of calcium, phosphorus, magnesium and creatinine in the serum, the hematocrit and hemoglobin. The development of hypocalcemia, hypophosphatemia, or hypomagnesemia require a short-term corrective therapy. Prior to administration of each dose is necessary to determine the level of serum creatinine.

Patients with the SRC prior to treatment should be carried out adequate rehydration. Loop diuretics combined with zoledronic acid with care (may develop hypocalcaemia), and only after a sufficient hydration.

**Table 1. The radial load on the authorities and all the patient's body using a drug 99mTc-ZA**

Organ	The dose (Rad / MBq)
Stomach	$1.5 \times 10^{-4}$
Lungs	$3.8 \times 10^{-4}$
Red bone marrow	$1.0 \times 10^{-3}$
ovaries	$3.6 \times 10^{-4}$
Testes	$2.4 \times 10^{-4}$
Upper large intestine	$2.1 \times 10^{-4}$
Lower large intestine	$3.8 \times 10^{-4}$
Bladder	$2.3 \times 10^{-2}$
Liver	$2.3 \times 10^{-4}$
Thyroid	$1.5 \times 10^{-4}$
Breast	$8.5 \times 10^{-5}$
Bones	$7.0 \times 10^{-3}$
Leather	$1.0 \times 10^{-4}$
Small intestine	$2.4 \times 10^{-4}$
kidneys	$1.4 \times 10^{-4}$
Skeletal muscles	$2.1 \times 10^{-4}$
Pancreas	$2.1 \times 10^{-4}$
Spleen	$1.9 \times 10^{-4}$
All the body (remains)	$2.5 \times 10^{-4}$
A heart	$2.4 \times 10^{-4}$
Adrenal glands	$2.8 \times 10^{-4}$
Brain	$1.7 \times 10^{-4}$
gallbladder	$1.9 \times 10^{-4}$
Thymus	$1.3 \times 10^{-4}$
Uterus	$6.1 \times 10^{-4}$
The effective dose (mSv / MBq) - 0.0016	

**CONCLUSION**

The novel radiopharmaceutical, 99mTc-ZA, has observed very good radiolabelling efficiency. 99mTc-ZA also

detected very encouraging data of radiation dosimetry. 99mTc-ZA is suggested for wide application after passing animal and human clinical tested.

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