JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Radiosynthesis and biodistibution of ^{99m}Tc-Albumin-Dutasteride for Prostate scintigraphy

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ABSTRACT

The present study aimed to develop ^{99m}Tc labeled dutasteride radiopharmaceutical and evaluate its targeted uptake in the prostate of normal rats. The radio labelling was achieved by direct labelling procedure and the radio labelling efficiency was assessed by ITLC (Instant thin layer cheromatography which was found to be 63.18% for ^{99m}Tc- Dutasteride while an efficiency of 94.9% was achieved for ^{99m}Tc-Albumin-Dutasteride that was stable for upto 4 hours. The protein binding assessed for the radiocomplex ^{99m}Tc-Albumin-Dutasteride was found to be ≈ 74% suggestive of a slow rate of elimination from kidneys and reticuloendothelial system. Blood kinetics profile depicted peak activity at 2 min post injection that gradually declined over time indicating a low rate of elimination from the blood pool. Biodistribution studies performed post intravenous injection showed appreciable (specify percentage) percentage specific uptake in prostate at 2 hours and was stable upto 4 hours indicating selectivity of the ^{99m}Tc-albumin-dutasteride complex for prostate gland. In conclusion, ^{99m}Tc-Albumin-Dutasteride has the potential to be exploited for its role as a radionuclide imaging probe. Further studies are warranted with regard to its specificity towards prostatic lesions so as to establish a comparative analysis in prostate related pathologies.

KEYWORDS: radiolabeling; dutasteride; biodistribution

RUNNING TITLE: Pharmacological evaluation of ^{99m}Tc-Dutasteride in normal rats.

INTRODUCTION

Dutasteride is a synthetic 4-azasteroid compound that is a selective inhibitor of both the type 1 and type 2 isoforms of steroid 5α-reductase (5AR), an intracellular enzyme that converts testosterone to 5α-dihydrotestosterone (DHT). It is chemically designated as (5α,17β)-N-{2,5 bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide. Since, prostate cancer is known to grow in response to male hormones such as testosterone or DHT and dutasteride work by lowering the amount of DHT, it has the potential for lowering the risk of developing prostate cancer. Recently, dutasteride has been shown to reduce the risk of prostate cancers and precursor lesions and improved outcomes related to benign prostatic hyperplasia (4). Hence, it becomes all the more important to exploit the effectiveness of ^{99m}Tc labeled dutasteride as a radionuclide imaging probe for SPECT (Single Photon Emission Computed Tomography) imaging. As the drug is very much effective in benign prostatic hyperplasia, it has a potential to get localized in the hyperplastic sites of tumor and thus could be exploited for early diagnosis and staging of prostrate tumors. Therefore, the present study attempts to optimize labeling of dutasteride with ^{99m}Tc, perform initial physiochemical characterization and biodistribution in rats so as to propose its potential as a radionuclide imaging probe.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this study were of analytical grade. Stannous Chloride dihydrate was purchased from Sigma Aldrich Company and ITLC-SG strips was procured from MERCK. Technetium-99m pertechnetate ($^{99m}TcO_4^-$) was obtained from Post-Graduate Institute of Medical Education and Research (PGIMER) Chandigarh, India.

Animals

Male S.D rats (n=7) weighing 150-200g were procured from the Central Animal House, Panjab University, Chandigarh. The animals were housed in polypropylene cages in the departmental animal house under hygienic conditions and were acclimatized for atleast one week before putting them to lab conditions All the procedures were done in accordance with the standard guidelines for care and use of laboratory animals and the protocols followed were approved by the Institutional Animal Ethics Committee (IAEC), Panjab University, Chandigarh, India.

Radiolabeling

Labelling of Dutasteride was performed by Stannous Chloride dihydrate reduction method. ^{99m}Tc-Dutasteride was prepared by adding 200µCi of $^{99m}TcO_4^-$ to a vial containing 100µg of dutasteride (1mg/ml of absolute ethanol). To the above preparation, 50µg of $SnCl_2 \cdot 2H_2O$ (1mg/ml solution in 0.1N HCl) was added and the pH was adjusted to 7-7.5 with 0.1M NaHCO3. The contents were incubated for 1 hour for the completion of reaction (Table 1).

The radiolabelling efficiency achieved by direct labelling dutasteride with $^{99m}TcO_4^-$ was only 63.18% when subjected to ITLC so to enhance the efficiency, Dutasteride-albumin dimer was prepared before subjecting the drug to radiolabelling procedure. Optimal radiolabelling efficiency was achieved by standardization of the concentrations of BSA and reducing agent ($SnCl_2.2H_2O$). A mixture of dutasteride (1mg/ml in 12.5% ethanol) and albumin (1%BSA) was incubated for 30-45 mins in the ratio as shown in Table 2. This followed the addition of Stannous chloride dihydrate (50µg) andfinally the complex was radiolabelled by addition of 200µCi $^{99m}TcO_4^-$and was again incubated at room temperature for 1hr (Fig.1) . The labelling efficiency was assayed with the help of ITLC.

Radiochemical Purity

Percentage labeling of dutasteride with ^{99m}Tc was assessed by ascending chromatography technique. A single spot of the preparation was applied at the point of origin on ITLC strips of appropriate length and width. These strips were then placed in tubes containing 100% acetone to check for any dissociation or degradation of labelled complex and a mixture of Pyridine, Acetic acid and water in the ratio of 3:5:1.5 as mobile phases to measure the amount of Free $^{99m}TcO_4^-$ fraction and Hydrolysed ^{99m}Tc fraction respectively in the radiopreparation. The strips were then left undisturbed to allow movement of the mobile phase. When the solvent migrates to the marked end of the chromatographic paper, they were removed from the developing vials, air-dried and counted for activity at different sections in well-type gamma-sensitive probe (Nucleonix, India).

***In vitro* Serum Stability of Radiocomplex**

Blood samples were drawn from rats under light ether anesthesia by puncturing the retro-orbital plexus using sterilized glass capillaries. Serum was then collected for the serum stability analysis of the complex. Briefly, 100 µl of the radiocomplex (1 mCi) was incubated with 900 µl of serum at 37°C for different time intervals up to 6 hrs. Small aliquots were subjected to ITLC at different time intervals in 100% acetone to check for any dissociation or degradation of the labelled complex. The serum stability of the complex was evaluated in terms of % of radiolabelling efficiency (Fig.2).

Plasma Protein binding

This assay depicts the affinity of the drug for the proteins present in the plasma. 100 µl of the radiolabelled complex was incubated with 900 µl of plasma at 37°C for 1hr. After incubation, 1ml of 10% TCA was added to the mixture and centrifuged at 2500 rpm for 10 min.

Supernatant fraction (S1) was then collected in a different tube, and the pellet was resuspended in 1ml of 5% TCA and centrifuged again at 3000 rpm for 10 min. The supernatant (S2) was collected in a separate tube. Both the supernatant fractions thus obtained were combined and the radioactivity was measured in both the precipitate and supernatant fractions. Protein binding of ^{99m}Tc Dutasteride complex was expressed as % fraction of radioactivity bound to the protein to that of the total radioactivity.

$$\% \text{ Protein binding} = \frac{\text{Counts in the pellet X 100}}{\text{Total counts (S1+S2+Pellet)}}$$

Lipophilicity (Partition Coefficient measurement)

Lipophilicity is a measure of affinity of prepared radiolabelled complex towards aqueous or organic solvents. The partition coefficient was calculated in a mixture of organic and aqueous phases. To a mixture of 1.9ml normal saline and 2 ml of n-octanol, 100 µl of the prepared radiolabeled complex was added. The mixture was vortexed well and allowed to stand undisturbed for 30 minutes at room temperature. The aqueous and the organic phases were carefully separated and the radioactivity in each fraction was measured. Lipophilicity was calculated as the fraction of ^{99m}Tc dutasteride complex soluble in the organic phase.

$$\% \text{ Lipophilicity} = \frac{\text{Counts in organic phase X 100}}{\text{Total counts (ie. Organic + aqueous phase)}}$$

The partition coefficient (Po/w) was calculated on the basis of the ratio (activity in the *n*-octanol layer)/(activity in the aqueous layer) and is expressed as log Po/w.

Blood Kinetics

Blood clearance of ^{99m}Tc labeled dutasteride was studied in normal rats after intravenous administration of 200µCi of the prepared radiopharmaceutical. Blood samples were withdrawn at different time intervals with the help of sterilized glass capillaries by puncturing the ocular vein of the rats and the radioactivity in each sample was measured.Data was expressed as percentage of total injected activity per ml of blood (% specific activity) for n=3 (Fig.3).

Biodistribution

The distribution of the radiolabeled complex was determined by administering 200µCi of ^{99m}Tc labeled dutasteride intravenously into the penile vein of the anaesthesized rats. Animals were sacrificed at different time intervals (1 hr, 2 hr, 4hr) and the desired organs were removed.Each organ was weighed and counted usingNaI (Tl) scintillation counter and expressed as % injected dose per gram of tissue weight (Table 3).

Statistical analysis

Each experimental parameter was repeated three times and differences in the data were evaluated with one way analysis of variance (ANOVA) test. Results are reported as mean± standard deviation(SD). The level of significance was set at $P \leq 0.05$.

RESULTS

The present study evaluated the prostate specificity of ^{99m}Tc labelled dutasteride in normal rats. The radiolabelling efficiency achieved by direct labelling of dutasteride with $^{99m}TcO_4^-$ was only 63.18% when subjected to ITLC, therefore, to enhance the efficiency, Dutasteride-albumin dimer was prepared before subjecting the drug to radiolabelling procedure. The radiolabelled complex exhibited a high labelling efficiency of 94% that was sufficiently stable in *in vitro* conditions as a function of time up till 4 hours at room temperature. Further it was noticed that an incubation time of 1 hour for the reaction mixture yielded maximum efficiency which started degrading with time post 1 hour incubation. The plasma protein binding for the radiolabeled preparation was found to be 74.03± 3.052 %. Log P for the radiocomplex was observed to be -0.41 ± 0.28 and thus % lipophilicity calculated was in the range of 30-40%.

Blood kinetics profile depicted peak activity at 2 min post injection of the radiopcomplex that gradually decreased as a function of time. Biodistribution studies revealed maximum percentage specific activity in liver 2 hours post injection followed by lungs and spleen that remained unchanged even at 4 hours. A significant uptake was noticed in prostate at 2 hours and was stable upto 4 hours. An insignificant uptake was observed in thyroid and stomach whereas a substantial lung uptake was noticed at both 2 hours and 4 hours of study. A significant uptake in kidneys at 2hours followed by a significant drop/wash out in at 4 hours was also observed after administration of the radiocomplex.

DISCUSSION

The maximum radiolabelling efficiency achieved with direct radiolabeling of dutasteride with 99mTcO4$^-$ was 63.18% using stannous reduction method. $SnCl_2.2H_2O$ lowers the oxidation state of ^{99m}Tc that makes it chemically reactive and permits

it to form complexes with various chelating agents that have electron donating groups therefore, dutasteride having NH_2 donor groups in its structure which might have resulted in coordinate covalent bond formation with ^{99m}Tc. However, further confirmative studies are required to clearly support this assumption.

Since direct labelling yielded lower efficiency, therefore, dutasteride-albumin dimer was prepared before subjecting dutasteride to radiolabelling. Dutasteride albumin dimer was successfully labelled with $^{99m}TcO_4^-$ that yielded a high radiolabelling efficiency of 91-94%. The serum albumin is an endogenous nano-particle and is known for its binding properties to various endogenous metabolites, drugs and metal ions and therefore has been exploited in the present study for its usage as a ligand for indirect labelling. This approach enhanced the radiolabelling efficiency as albumin has ability to bind both to dutasteride as well as ^{99m}Tc. Further, it could provide a nanobased delivery system wherein albumin binds to albumin receptors known as (gp60) and can facilitate localization of the radionulclide as well as the drug to its target site. The accumulation of albumin in solid tumors forms the rationale for developing albumin-based drug delivery systems for tumor targeting and thus the developed radiocomplex is expected to have specificity for prostatic cancerous lesions.

The radiocomplex was sufficiently stable in *in vitro* physiological conditions for upto 4 hours at room temperature. Percentage protein binding of the radiopharmaceutical is an important parameter determining its biodistribution, target uptake and plasma clearance. A number of factors such as nature of the amino groups involved, charge on the radiopharmaceutical etc. have an influence on the interaction of the radiolabelled complex with the plasma proteins (mainly albumin, globulin)[18,19] . The protein binding of ^{99m}Tc-dutasteride albumin complex assessed in serum was found to be 74.03 %± 3.052 % suggesting a low rate of elimination via kidneys and reticuloendothelial system owing to high protein bound fraction.

Lipophilicity is a key physicochemical property contributing to the ADMET characteristics of drugs (permeability through membranes impacting their pharmacokinetics) and thus determines the overall quality of candidate drug molecules[20]. Dutasteride in itself is hydrophobic in nature, however the complete dissolution of dutasteride-albumin dimer in ethanol imparts hydrophilic nature to the complex as suggested by log P value of -0.41 ± 0.28.

Blood kinetics determined the rate at which the radiopharmaceutical cleared from the blood circulation. The peak activity was observed at 2min post injection with sharp decline up till 10 min followed by a slow gradual decrease up till 1 hour. The blood pharmacokinetics of the radio complex showed a typical biphasic clearance of the radio complex. A fast clearance phase was observed after 2 min of administration of the radio complex followed by a slow clearance phase after 10 min post administration. The first peak at 2 min indicates fast clearance from the blood suggesting that the radio complex is quickly taken up by different organs or is being eliminated from the body rapidly. The slow clearance of tracer observed in the second phase of blood kinetics is suggestive of the slow release of the tracer from different organs into the systemic pool.

The biodistribution pattern was observed in rats after intravenous administration of ^{99m}Tc- albumin-dutasteride trimer in the penile vein of rats. The maximum percentage activity per gram of tissue after 2 hour post injection was observed in liver followed by spleen, lungs and kidneys. Since dutasteride gets extensively metabolized in liver to inactive metabolites, which are eliminated through the bile and urine , this could have possible to corroborated well with increased percent specific uptake of radio complex in liver and spleen. Kidneys also showed retention of the radiocomplex even after 4 hours, indicating the excretion of activity through renal route. Further, the delayed clearance of the radio complex can be well corroborated from the

present finding of high protein binding of the ^{99m}Tc-albumin-dutasteride. Accumulation in lungs could be attributed to ^{99m}Tc labelled albumin aggregates during the preparation of formulation and got trapped in pulmonary capillary bed. This could be avoided using appropriate size millipore filters in future preparations. The present study also witnessed specific uptake of the radiocomplex in rat prostate 2 hrs post injection which did not show significant change up to 4 hours. An insignificant uptake in thyroid and stomach is indicative of appreciable degree of radiolabelling efficiency.

In conclusion, the study effectively labeled dutasteride with ^{99m}Tc using albumin as linker and nano based delivery system and the radiocomplex exhibited desired stability both in *in vitro* and *in vivo* conditions. Further, the biodistribution pattern suggests that ^{99m}Tc-albumin-dutasteride gets cleared by hepatobiliary route and possesses selectivity towards the prostate tissue and it shall be worthwhile exploring it further for its enhanced specificity for prostatic cancerous lesions so as to establish a comparative analysis in prostate related pathologies and its potential in radionuclide imaging.

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ACKNOWLEDGEMENT:

The authors acknowledge with thanks staff of Post Graduate Institute or Medical Education and Research (PGIMER) Chandigarh, India for providing necessary material for the study. The financial support by the Department of Department of Science and Technology (DST), New Delhi is gratefully acknowledged.

Table legends:

Table I - Direct Labelling of Dutasteride by Stannous Chloride dihydrate reduction method

Table II- Indirect Labelling of Dutasteride using Albumin as linker.

Table III- Biodistribution ^{99m}Tc- albumin dutasteride in normal rats

Figure legends:

Figure I: Effect of reaction time on the labelling efficiency of ^{99m}Tc-Albumin–Dutasteride.

Figure II: Invitro Serum stability of ^{99m}Tc-albumin-dutasteride following ITLC in acetone at different intervals of time

Figure III: Blood Kinetics of ^{99m}Tc-albumin-dutasteride following intravenous injection in rats

Stannous(µg)	Dutasteride (µg)	Activity ((µCi)	% Labeling efficiency (bound)
10	10	200	54.33
10	20	200	24.89
10	50	200	52.75
10	100	200	44
50	50	200	35.6
50	**100**	**200**	**63.18**
100	100	200	64.9
100	200	200	56.05
200	200	200	41.22
200	400	200	53.14
300	600	200	61.5

Table I: Direct Labelling of Dutasteride by Stannous Chloride dihydrate reduction method

	Albumin (µg)	Stannous	Activity ((µCi)	% Labeling efficiency (bound)
100	50(0.5%)	50	200	81.22
100	100 (0.5%)	50	200	83.49
100	50 (0.5%)	100	200	89.35
100	100(0.5%)	100	200	83.83
100	**50 (1%)**	**50**	**200**	**94.95**
100	100 (1%)	50	200	91.03
100	50(1%)	100	200	89.46
100	100(1%)	100	200	92.73

Table II- Indirect Labelling of Dutasteride using Albumin as linker

Table III- Biodistribution ^{99m}Tc- albumin dutasteride in normal rats

	SP.UPTAKE (2Hrs)	SP.UPTAKE (4Hrs)
Kidney	9.300± 2.722	3.832 ± 1.013
Stomach	0.244±0.139	0.168±0.024
Small Intestine	0.647±0.186	1.685±0.340
Large Intestine	0.626±0.241	2.178±0.177
Spleen	17.980±6.660	11.480±3.434
Heart	0.575±0.112	0.751±0.074
Thyroid	0.460±0.076	0.782±0.112
Bladder	3.783±2.150	2.414±1.100
Prostate	**1.644±0.377**	**1.979±0.723**
Brain	0.275±0.154	0.156±0.078
Lungs	15.280±6.750	14.240±5.850
Liver	35.320±4.560	33.460±7.650

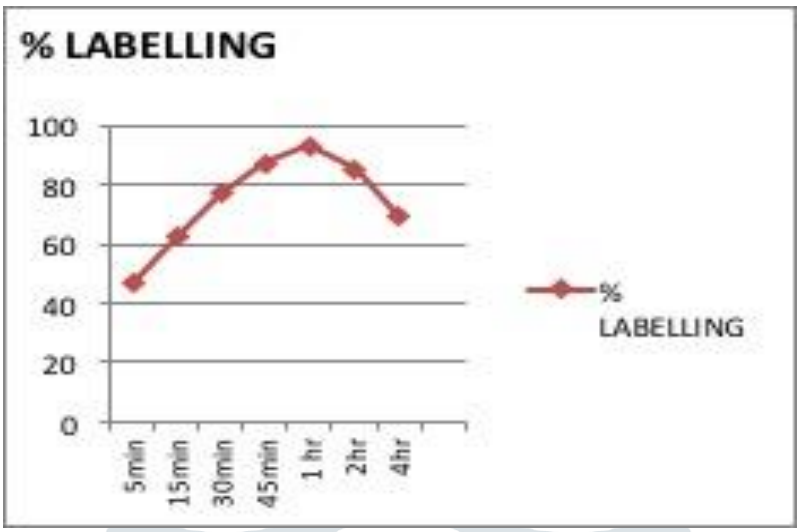

Fig. I :Effect of reaction time:

Fig.II: Invitro Serum stability of ^{99m}Tc-albumin-dutasteride

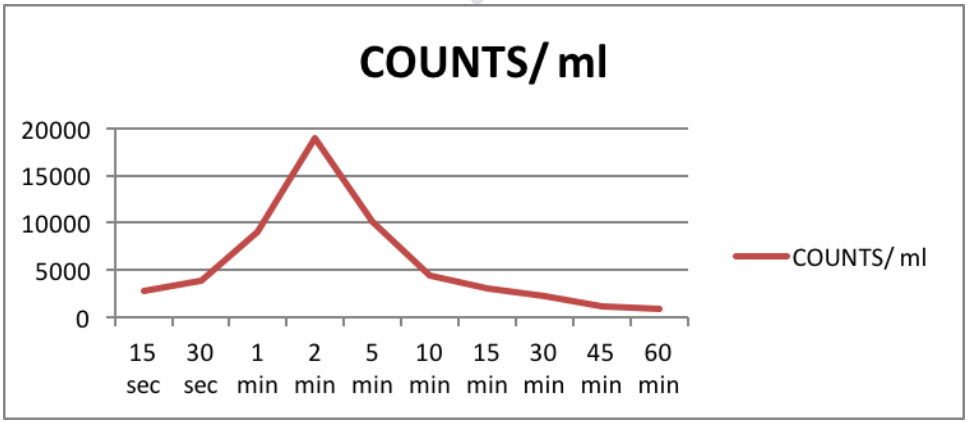

Fig.III: Blood Kinetics of ^{99m}Tc- dutasteride albumin following intravenous injection in rats