

# Preparation and Biodistribution of [<sup>67</sup>Ga]-labeled-oxytocin for SPECT purposes

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**Background:** Oxytocin (OT) is a paracrine hormone with various biological activities and many sex organs in both sexes, as well as many tumor cells have shown to have related receptors. In this study the development of a receptor imaging tracer for possible tumor imaging has been described. **Materials and Methods:** OT was successively labeled with [<sup>67</sup>Ga]-gallium chloride after conjugation with freshly prepared cyclic DTPA-dianhydride. The best results of the conjugation were obtained by the addition of 1 ml of a OT pharmaceutical solution (2 mg/ml, in phosphate buffer, pH=8) to a glass tube pre-coated with DTPA-dianhydride (0.02 mg) at 25 °C with continuous mild stirring for 30 min. Radiochemical purity (RCP) of the labeled compound was determined, using RTLC and ITLC followed by stability tests and animal biodistribution studies. **Results:** Radiolabeling took about 60 minutes with a RCP higher than 98 % at optimized conditions (specific activity = 1000 Ci/mM, labeling efficiency 80%). The stability of the tracer at room temperature was significant, up to an hour. Preliminary *in vivo* studies in normal female rat model showed ovary/blood and ovary/muscle ratio uptake of the tracer in 60 minutes to be 4.53 and 9.18, respectively. The result was consistent with the reported OT receptor distribution in normal female mammals. **Conclusion:** The radiolabeled oxytocin, prepared in this study, was a possible fast acting tracer for OT receptor imaging; studies however, more studies are required to determine the best imaging conditions especially in larger mammal animals. *Iran. J. Radiat. Res., 2009; 7 (2): 105-111*

**Keywords:** Radiogallium, oxytocin, radiolabeling, biodistribution, radiotracer, cyclotron.

## INTRODUCTION

Oxytocin (OT) is a nonapeptide hormone having potent and specific contractile effects on the parturient myometrium, and it was originally characterized as a hormone with a permissive role in female reproduction: facilitating uterine contraction and milk ejection <sup>(1, 2)</sup>. In males,

OT was found to be one of the most potent agents to induce penile erection by electrical or pharmacological stimulation of paraventricular nuclei in rats, rabbits and monkeys <sup>(3)</sup>. Nevertheless, an additional and peripheral role for OT has been proposed. OT mRNA is synthesized within intrauterine tissues during late gestation in both rats <sup>(4, 5)</sup> and humans <sup>(6)</sup>.

Interestingly, the presence of the oxytocin receptor (OTR) gene and protein in rabbit and human cavernous tissue in a similar concentration to that found in other portions of the male genital tract has been reported <sup>(7)</sup> classically considered the main male target of OT, such as the epididymis <sup>(8)</sup>. In the epididymis, OTR mediates an increase in both *in vitro* and *in vivo* contractility and sperm output.

Various radiolabeled OT compounds have been prepared and employed in research studies. For instance, using <sup>3</sup>H-labeled OT, specific binding sites for OT were localized in various areas of the brain of adult male guinea pigs by autoradiography method <sup>(9)</sup>. The brain metabolism of OT by peptidases has been studied using [<sup>14</sup>C] oxytocin labeled at either the tyrosine-2 or the glycine-9 residue <sup>(10)</sup>. In order to study the presence of OT receptors in rat penis, Zhang *et al.* used <sup>125</sup>I-OT in their studies <sup>(11)</sup>. But, according to our knowledge no radiolabeled OT has ever been reported for ultimate use in imaging studies using

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SPECT and/or PET methods.

There are various tissues expressing oxytocin receptors such as uterus<sup>(12)</sup>, ovary and corpus luteum<sup>(13)</sup>, prostate, testis<sup>(14)</sup> etc. Notably, it has been reported in many breast cancers oxytocin receptor to be over-expressed. Antiproliferative effects of OT have also been observed in various breast carcinoma cell lines, as well as in human neuroblastoma and astrocytoma cells, suggesting the existence of oxytocin receptors on these malignancies<sup>(15, 16)</sup>.

In order to obtain an OT conjugate to use in diagnostic studies with metallic SPECT radioisotopes, <sup>67</sup>Ga-labeled OT was prepared for preliminary biodistribution studies, based on the recent experiences on the preparation of radiometal-labeled proteins<sup>(17, 18)</sup>.

A precise labeling strategy was employed with various OT concentrations using available gallium-67. Finally, the stability and biodistribution of radiolabeled oxytocin conjugate were determined, using *in vitro* and *in vivo* experiments.

## MATERIALS AND METHODS

Production of <sup>67</sup>Ga was performed at the Agriculture, Medicine and Industrial Research School (AMIRS), 30 MeV cyclotron (Cyclone-30, IBA). Enriched zinc-68 chloride with enrichment of >95% was obtained from Ion Beam Separation Department at NRCAM. Sephadex G-50, sodium acetate, phosphate buffer components methanol and ammonium acetate were purchased from Sigma-Aldrich Chemical Co. (U.K.). Oxytip™ was a pharmaceutical sample of oxytocin purchased from Abourayhan Pharmaceutucal Co. (Tehran, Iran) and was used without further purification. Radio thin layer chromatography (RTLC) was performed by counting different 5 mm slices of polymer-backed silica gel paper and/or Whatman thin layer sheets, using a thin layer chromatography scanner, Bioscan AR2000, Bioscan Europe Ltd., Paris, France. The area under the curve of 184 keV

(a major photopeak for <sup>67</sup>Ga) of each animal tissue sample was calculated using a high purity germanium (HPGe) detector coupled with a Canberraä (model GC1020-7500SL) multichannel analyzer. All values were expressed as mean ± standard deviation (Mean± SD), and the data was compared using student T-test. Statistical significance was defined as P<0.05. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edn.

### Production of <sup>67</sup>Ga

<sup>68</sup>Zn(p,2n)<sup>67</sup>Ga was used as the best nuclear reaction for the production of <sup>67</sup>Ga. Impurities could be removed in the radiochemical separation process. After the target bombardment process, chemical separation was carried out in no-carrier-added form. The irradiated target was dissolved in 10 M HCl (15 ml), and the solution was passed through a cation exchange resin (AG 50W, H<sup>+</sup> form, mesh 200-400, h:10 cm, Ø:1.3 cm) which had been preconditioned by passing 25 ml of 9 M HCl. The column was then washed by 25 ml of 9M HCl at a rate of 1 ml/min to remove copper and zinc ions. To the eluent 30 ml water plus about 100 ml of a 6 M HCl solution was added. The latter solution was loaded on another exchange resin (AG1X8 Cl<sup>-</sup> form, 100-200 mesh, h: 25 cm, Ø:1.7 cm) pretreated with 6 M HCl (100 ml). Finally, the gallium-67 was eluted as [<sup>67</sup>Ga] GaCl<sub>3</sub> using 2 M HCl (50 ml); the whole process took about 60 min.

### Control of Radionuclide purity

Gamma spectroscopy of the final sample was carried out counting in a HPGe detector coupled to a Canberraä multichannel analyzer for 1000 seconds.

### Chemical purity control

The presence of zinc and copper cations were checked by polarography method. Even at 1 ppm of standard zinc and copper concentrations, the areas under the curve of

polarogram of the test samples were lower than the standards.

### **Conjugation of cyclic DTPA di-anhydride with human recombinant OT**

The chelator diethylenetriamine penta-acetic acid dianhydride was conjugated to the OT, using a small modification of the well-known cyclic anhydride method <sup>(19)</sup>. Conjugation was performed at a 1:1 molar ratio. In brief, 20  $\mu\text{l}$  of a 1 mg  $\text{ml}^{-1}$  suspension of DTPA anhydride in dry chloroform (Merck, Darmstadt, Germany) was pipetted under ultrasonication and transferred to a glass tube. The chloroform was evaporated under a gentle stream of nitrogen. Commercially available OT (5 mg, 0.5 ml, pH 8) was subsequently added and gently mixed at room temperature for 60 min. Conjugation mixture was then passed through a Sephadex G-50 column (2' 15 cm, 2 g in 50 ml of Milli-Q<sup>®</sup> water) separately and one-milliliter fractions were collected and checked for the presence of protein, using UV absorbance at 280 nm or visible folin-phenol colorimetric assay. The fractions containing the highest concentration of the immunoconjugate were chosen and kept at 4°C and for radiolabeling.

### **Radiolabeling of OT conjugate with $^{67}\text{Ga}$**

The OT conjugate was labeled using an optimization protocol according to literature <sup>(20, 21)</sup>. Typically, 37-40 MBq of  $^{67}\text{Ga}$ -chloride (in 0.2M HCl) was added to a conical vial and dried under a flow of nitrogen. To the Ga containing vial, conjugated fraction was added in 1 ml of phosphate buffer (0.1 M, pH= 8) and mixed gently for 30 seconds. The resulting solution was incubated at room temperature for 30 minutes. Following the incubation, the radiolabeled OT conjugate was checked using ITLC/RTLC methods for the purity. In case of significant presence of impurities the sample can be purified using gel filtration as described above. Control labeling experiments were also performed using  $^{67}\text{GaCl}_3$ , and DTPA with  $^{67}\text{GaCl}_3$ . Both reaction mixtures were passed through

separate gel filtration columns and eluted with PBS. In case of gel filtration fraction which showed the presence of protein were used in the other experiments (n=3).

### **Quality control of [ $^{67}\text{Ga}$ ]-OT**

Paper chromatography: A 5 ml sample of the final fraction was spotted on a chromatography paper (Whatman No. 1. Whatman, Maidstone, UK), and developed in a mixture of 1mM DTPA in  $\text{DDH}_2\text{O}$  as the mobile phase.

### **Stability testing of the radiolabeled compound**

Stability of  $^{67}\text{Ga}$ -DTPA-OT in PBS was determined by storing the final solution at 4°C for 24 hours and performing frequent ITLC analysis to determine radiochemical purity. Frequent ITLC analysis was performed. ITLC analysis of the conjugated product was performed to monitor degradation products or other impurities. After subsequent  $^{67}\text{Ga}$ -labelling of the stored conjugated product, labeling efficiency and radiochemical purity were both determined.

### **Stability testing of the radiolabeled compound in presence of serum**

Labeled compound stability in serum was assessed by gel filtration on a Sepharose column (1 × 30 cm). The column was equilibrated with PBS and eluted at a flow rate of 0.5 mL/min at room temperature; 0.5 mL fractions were collected.

### **Biodistribution of $^{67}\text{Ga}$ -DTPA-OT in normal female rats**

To determine its biodistribution,  $^{67}\text{Ga}$ -DTPA-OT was administered to normal female rats. A volume (50 ml) of final  $^{67}\text{Ga}$ -DTPA-OT solution containing  $40 \pm 2$  mCi radioactivity was injected intravenously to rats through their tail vein. The animals were sacrificed at exact time intervals (30 and 60 min), and the specific activity of different organs was calculated as percentage of urea under the curve of 184 keV peak per gram using an HPGe detector.

## RESULT AND DISCUSSION

### Conjugation of OT with DTPA cyclic di-anhydride and radiolabeling of OT with $^{67}\text{Ga}$

Oxytocin has a molecular mass of 1007 daltons and according to many vendor's descriptions one international unit (IU) of oxytocin is equivalent to about 2 micrograms of pure peptide. The peptide has been reported to have a 1-2 minutes biological half life in serum and 28 minutes in brain fluids <sup>(22, 23)</sup>. These conditions can be challenging for the development of a possible tracer since the low molecular weight can be a problem in separation of the conjugated peptide from DTPA molecules. On the other hand, low biological half life can diminish the uptake ratio in target tissue while a rather long physical half life (72 hours for Ga-67) can partially compensate for this low biological stability.

The labeling yield of  $^{67}\text{Ga}$ -DTPA-OT has been studied in the wide range of OT/DTPA ratios in order to optimize the process and to improve  $^{67}\text{Ga}$ -DTPA-OT performance in vitro. The overall radiolabeling efficiency was over 77-80%, and the specific activity was kept in the range of 1000 Ci/mM. Due to the presence of disulfide bond in the structure, OT is exceedingly sensitive to external influences. Such influences include heat and alkali sensitivity, sensitivity with respect to oxidizing and reducing agents, as well as to strong acidly reacting substances. Thus, radiolabeled OT using  $^{99\text{m}}\text{Tc}$  and radioiodine that mostly benefit from oxido/redox reactions always impose risk of biological activity loss. The only possible conjugating site for an anhydride-containing BFL (namely, ccDTPA) is the free amino group in cysteine moiety as shown in figure 1.

The conjugated DTPA-Oxytocin fractions containing the maximum protein content were mixed with  $^{67}\text{Ga}$ -GaCl<sub>3</sub> solution, vortexed and kept at room temperature. Small fractions were taken from this mixture and tested by RTLC to find the best time scale for labeling. After

an hour, free  $^{67}\text{Ga}$ /conjugated  $^{67}\text{Ga}$  ratio in the labeled sample remained unchanged. The mixture was then passed through another Sephadex G-50 gel filtration column in order to remove trace amounts of unbound  $^{67}\text{Ga}$  cation.

The eluted fractions were checked by Folin-Colcitateau<sup>0</sup> reagent, and for presence of radioactivity in order to determine the  $^{67}\text{Ga}$ -DTPA-OT containing fractions. The fraction with the maximum absorbance using folin method, which consisted of the maximum radioactivity, was chosen as the suitable final product with appropriate specific activity for animal tests. The radiolabeling reached to 90% after 60 min. Figure 2 demonstrates the RTLC scheme of free Ga<sup>3+</sup>, Ga-DTPA. Due to high K<sub>d</sub> for Ga-DTPA complex any free Ga<sup>3+</sup> cation can be eluted by a 10 mM DTPA solution (pH. 5.5) when used as RTLC eluent and the resulting R<sub>f</sub> for free Ga and Ga-DTPA species is 0.9 (figure 3).

On the other hand, the radiolabeled peptide retains at the origin of the stationary phase (R<sub>f</sub>. 0.0) used (Silica or Whatman) when sampled. Thus, the best eluent for discrimination between the free Ga/GaDTPA from radiolabeled peptide was shown to be 10 mM DTPA. Various other eluents were also used for TLC studies, but the best reproducible data was obtained from above mentioned mobile phase (data not shown).

### Stability of radiolabeled peptide in vitro

The stability of the radiolabeled

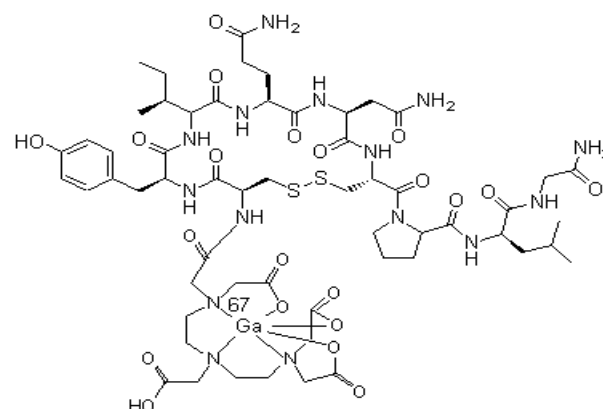


Figure 1. Possible chemical structure of  $^{67}\text{Ga}$ -DTPA-Oxytocin.



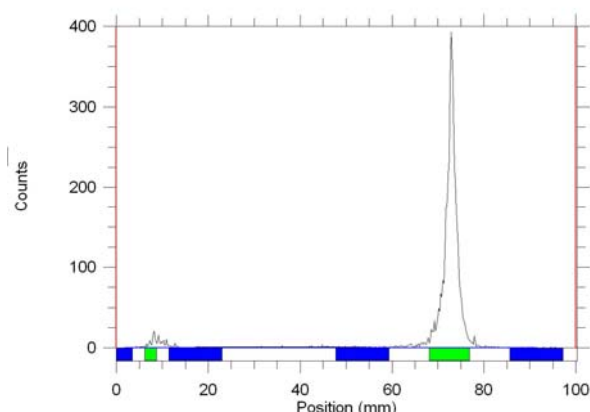


Figure 2. RTLC of  $^{67}\text{Ga}$  and  $^{67}\text{GaDTPA}$  in 10 mM DTPA in  $\text{DDH}_2\text{O}$  as mobile phase and Si stationary phase.

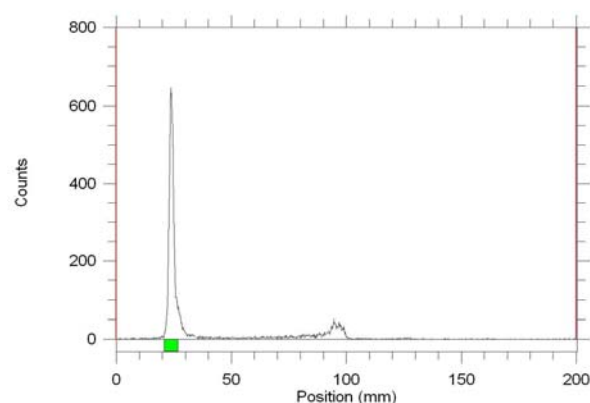


Figure 3. RTLC of  $^{67}\text{Ga}$ -DTPA-OT in 10 mM DTPA in  $\text{DDH}_2\text{O}$  as mobile phase.

peptide *in vitro* was determined after challenge with phosphate-buffered saline and serum. ITLC analysis showed that the peptide was broken down after 1 hour, indicating that the Ga-peptide chelate was of low stability at room temperature.

#### Stability of radiolabeled peptide in presence of serum *in vitro*

Using gel filtration chromatography after incubation of  $[^{67}\text{Ga}]$ -DTPA-OT with PBS diluted human serum for 2 h, almost all of the radioactivity had eluted in the same position as  $[^{67}\text{Ga}]$ -DTPA; there was no sign of  $^{67}\text{Ga}$ -DTPA-OT detectable in presence of human serum.

#### Biodistribution studies

The distribution of  $[^{67}\text{Ga}]$ -DTPA-OT among tissues were determined in normal rats. A volume (0.1 ml) of final  $[^{67}\text{Ga}]$ -DTPA-

OT solution containing 4.4-5.2 MBq radioactivity was injected into the dorsal tail vein. The total amount of radioactivity injected into each mouse was measured by counting the 1-ml syringe before, and after injection in a dose calibrator with a fixed geometry. The animals were sacrificed by ether asphyxiation at selected times after injection (30 and 60 minutes), and the tissues (blood, heart, spleen, kidneys, skull, fat, brain, bladder, breasts, liver, uterus, ovaries, stomach, lung, skin) and feces were weighed to determine their specific activities with a recently calibrated HPGe detector as a percent of area under the curve of 184 keV per gram of tissue (figure 4).

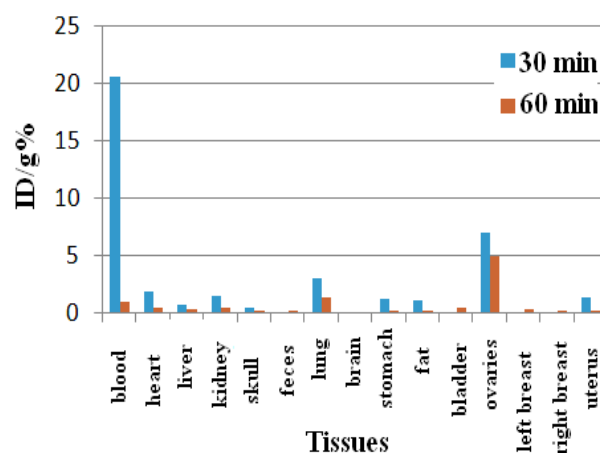


Figure 4. Bio-distribution of  $^{67}\text{Ga}$ -DTPA-oxytocin in various female rat organs 30 and 60 minutes post-injection calculated by ID/g% based on the area under the curve of 184KeV.

#### CONCLUSION

The radioactivity was rapidly washed out from blood circulation due to tissue uptake or fast kidney excretion possibly between 30 to 60 minutes. The best target tissue was shown to be ovaries containing 6-7% of total injected activity; however, one can't be sure if 30 minutes can be the optimum time for tissue uptake. This observation has been in accordance with the reported OT receptor biodistribution in ovaries (13). Although a high ovarian uptake was observed at 30 minutes post injection (7%), a higher blood activity resulted in a weak ovary/blood ratio (0.34), while at 60

minutes ovary/blood ratio was satisfactorily, 4.53 suggesting 60 minutes would have been a suitable time for *in vivo* imaging studies. No brain uptake was observed due to the water solubility of the peptide and low blood brain barrier permeability.

Breasts did not show any significant uptake and that was not unpredictable since the OT receptor are available on this tissue at breast-feeding period, according to the ethical rules in the institution do experiments on breast-feeding animals.

The uterus uptake was not also significant, and most of other reports demonstrated OT receptors to be more predominant near the partum or post-partum periods. Lung uptake could be resulted by natural free gallium uptake of the tissue and/or a result of high peptidase enzyme concentrations leading to radio-labeled peptide breakdown. However, there were interesting reports on the existence of OT receptors on some lung tumors due to positive response of oxytocin therapy in SCLC malignancies via inhibition of endothelial mitosis <sup>(24)</sup>.

A more detailed study on this radio-tracer is suggested, using MCF-7 or other breast cancer cell lines, as well as SPECT imaging studies in a bigger mammalian model in order to demonstrate the imaging value of the tracer more clearly.

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