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# Synthesis and biodistribution study of a chlorotoxin derivative peptide labeled with 131-iodine for tumor therapy

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***Background:*** Chlorotoxin is a 36-amino acid peptide found in the venom of the *Leiurus quinquestriatus* which blocks small-conductance chloride channels. Chlorotoxin binds preferentially to glioma cells that allow development of new methods for the treatment and diagnosis of several types of cancer. Thus chlorotoxin derivative was labeled with $^{131}$I for further investigation. ***Materials and Methods:*** A chlorotoxin derivative was synthesized on a solid phase using a standard Fmoc strategy. Labeling with iodine-131 was performed through chloramine-T method and radiochemical analysis involved sephadex G-25 and HPLC methods. The stability of radiopeptide was checked in the presence of PBS and human serum at 37 °C up to 24 h. The biodistribution was studied in mice. ***Results:*** The chemical purity of synthesized peptide as assessed by analytical RP-HPLC was 95%. Labeling of peptide resulted in a radiochemical yield of 80% with radiochemical purity of > 95% with specific activity of 0.740 GBq/μmol. Result of *in vitro* studies demonstrated acceptable stability of compound in human serum and PBS solution. Biodistribution data showed moderate blood clearance, with concentration of radioactivity in the kidneys, liver, intestine and stomach. ***Conclusion:*** Results indicates that the labeled Chlorotoxin derivative might be useful in determining tumor extent and also, tumor therapy of gliomas or possibily other cancers. **Iran. J. Radiat. Res., 2011; 8(4): 243-248**

***Keywords:*** *Cancer, peptide, $^{131}$I, labeling, chlorotoxin.*

## INTRODUCTION

Chlorotoxin is a peptide derived from the venom of the scorpion *Leiurus Quinquestriatus* which have four disulfide bonds and a single tyrosine residue. This neurotoxin can be produced and purified from *E. coli* by recombinant DNA and protein purification techniques. A synthetic peptide version of chlorotoxin (TM-601) is available commercially [(1)].

It was reported that the crude venom, extracted from the scorpion *Leiurus quinquestriatus* inhibits small-conductance of $Cl^-$ channels isolated from rat brain [(3)]. Chlorotoxin is the first reported high-affinity peptide ligand for $Cl^-$ channels and it blocks small conductance chloride channels. Each chloride channel can be closed by only one ligand molecule [(2,3)]. Using a recombinant chlorotoxin it was demonstrated that chlorotoxin specifically and selectively interacts with matrix metalloproteinase-2 (MMP-2) isoforms which are specifically unregulated in gliomas and related cancers, but are not normally expressed in brain [(4-11)].

Gliomas are among the most deadly forms of cancer for which effective treatment strategies are currently lacking. Indeed, despite the overall advances in chemo- and radiation-therapy regimens, the median survival of glioma patients has been unaltered in the last 20 years [(12-14)]. This lack of success in treatment of gliomas seems to be due in part to their high resistance to radiation and chemotherapy but additionally to their unusual ability to disperse and invade healthy brain tissue. Because of the lack of glioma specific markers, unequivocal diagnosis of gliomas requires tissue biopsy and relies primarily on histopathological criteria. Correlation

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between glioma chloride channels (GCC) expression and tumor suggests that GCC may be a candidate protein to serve as a glioma-specific marker and may be useful for diagnostic and therapeutic purposes [14].

Chlorotoxin has been explored as a candidate for targeting gliomas [15-17]. This peptide can cross blood-brain and tissue barriers [14, 15] and binds to a phosphatidyl inositide, a phosphorylated lipid on lamellipodia of tumor cells [18] Preclinical and clinical studies demonstrated the stability, safety, and efficacy of radioiodinated chlorotoxin [15].

In the present study we have synthesized a chlorotoxin derivative and have attached Iodine-131 to the molecule that subsequently allowed its detection. We evaluated radiochemical and biological characteristics of this iodinated derivative to be used as a molecule with diagnostic and therapeutic potential.

## MATERIALS AND METHODS

All chemical materials were purchased from commercial sources and used without additional purification. Rink amide MBHA (4-Methylbenzhydrylamine) resin and all of the Fmoc-protected amino acids were commercially available from NovaBiochem (Germany).

The reactive side chains of the amino acids were masked with one of the following groups: Arg, 2, 2, 4, 6, 7-pentamethyldihydrobenzofurane-5-sulfonyl; Asn, triphenylmethyl; Gln, triphenylmethyl; Lys, t-butoxycarbonyl; Tyr, t-butyl; Thr, t-butyl; Cys, acetamidomethyl; Asp, t-butoxy; His, trityl; . For sterility filtration, 20-µm Millex-GS filters from Millipore were used. Sodium Iodine ($^{131}$I) obtained from Radioisotope Group, AEOI (Atomic Energy Organization of Iran).

Analytical reverse phase-high performance liquid chromatography (RP-HPLC) was performed on a JASCO 880-PU (Japan) intelligent pump HPLC system equipped with a multiwavelength detector and a flow-through Raytest-Gabi γ-detector. CC250/4 Nucleosil 120-3C18 column from Macherey-Nagel were used for HPLC. The gradient systems consisted of 0.1% trifluoroacetic acid (TFA)/water (solvent A), acetonitrile (solvent B), flow: 1 mL/min, λ = 280 nm. Quantitative gamma counting was performed on ORTEC Model 4001 M (England) γ-system well counter.

### *Synthesis*

The peptide was synthesized by standard Fmoc solid-phase synthesis on Rink amide MBHA resin with substitution of 0.69 mmol/g. Coupling of each amino acid was performed in the presence of 5 molar excess of Fmoc-amino acid, 5 molar excess of Nhydroxybenzotriazole (HOBT), 5 molar excess of diisopropylcarbodiimide (DIC), and 5 molar excess of N-etthyldiisopropylamine (DIPEA) in N-methylpyrrolidone (NMP) for 2 h. Completeness of coupling reactions was monitored by the Kaiser test and the Fmoc groups were removed by adding 20% piperidine/N,N-dimethylformamide (DMF). After deportection and precipitation, the products were purified by RP-HPLC.

### *Labeling and radiochemical analysis*

Labeling of product was performed by chloramine-T method. Peptide (40 µg) was dissolved in PBS buffer (50 µl, 0.25 M, pH=7.5) then was added to a solution of 200 µci Na$^{131}$I (in 0.1 N NaOH), followed by 50 µl chloramine-T (4 mg/ml in PBS 0.05 M, pH=7.5). The component was mixed by a shaker for 3 min and the reaction was terminated by 100 µl sodium metabisulfite to suppress oxidation reaction (2.5 mg/ml in PBS 0.05 M, pH=7.5). For more purification labeled peptide was loaded on a gel chromatography (sephadex G-25) column and 1 ml fractions were collected. All those volumes corresponding to labeled peptide fraction were mixed. After reducing the volume, radiochemical yield of labeled peptide was determined by RP-HPLC on C18 column.

### *Stability study*

A volume of 1 ml (200 µci) of the labeled compound was incubated at 37°C with 1 ml



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of fresh human serum. Radiochemical stability was determined by taking samples of 10 µl at different times up to 24 h for analysis by sephadex G-25 column. Stability in PBS was also determined by incubation of 50 µl of the labeled compound with 1 ml PBS solution storing in room temperature for 24 h.

### *Animal studies*

Animal experiments were performed in compliance with the regulations of nuclear science and technology research institute (NSTRI), and with generally accepted guidelines governing such work. Male mice, weighing 25-30 g, were injected with 0.37 MBq of radiolabeled peptide in saline into the tail vein. For *ex vivo* counting, mice were sacrificed after 1, 4 and 24 h and various organs were dissected, weighed and counted for radioactivity. Data were expressed as the percentage of injected dose per gram of tissue (%ID/g).

## RESULTS AND DISCUSSION

A chlorotoxin derivative with sequence of M-C-M-P-C-F-T-T-D-H-Q-M-A-R-K-C-D-D-C-C-G-G-K-G-R-G-K-C-Y-G-P-Q-C-L-C-R, without disulfide bounds was synthesized by Fmoc strategy with an overall yield of nearly 50% (figure 1). The purity was 95% as confirmed by RP-HPLC method (figure 2). Labeling was performed by the electrophilic substitution of iodine-131 in the tyrosine ring using chloramine-T as the oxidant. After purification by sephadex G-25 column, the overall radiolabeling efficiency was about 80% at a specific activity of 0.740 GBq/µmol (figure 3). For collected labeled compound fractions radiochemical purity was >95% as obtained by RP-HPLC. The elution times were 3.44 min for free iodine and 14.56 min for $^{131}$I-peptide (figure 4). After 24 h incubation in human serum and PBS the radiochemical purity remained >57% and >65%, respectively.

A number of common methods are available for radioiodination of peptides. The choice of a radioiodination method depends on the structure of the peptide. Peptides that have an unsubstituted tyrosyl or histidyl residue available for oxidative iodination can be iodinated using the chloramines-T method [19]. This is an oxidative method that involves reacting chloramines-T (p -toluene sulfonochloramide) with $^{131}$I. For peptides which their structure is not consisted of tyrosine or histidine, or in which labeling of these amino acid residues may interferes with biological activity, $^{131}$I may be added onto primary amino groups (lysine residues and the peptide N-terminus) by using Bolton-Hunter reagent. This is a non oxidative procedure that can be used for peptides that contain methionines or other residues which that must remain in a reduced state to be bound to their respected receptors. Another method that is less likely to oxidize methionines or other residues in the molecule is the use of iodogen reagent. Although iodination of tyrosine or histidine residues using iodogen is one of the most convenient method, but employing chloramine-T method may give higher recoveries when working with small quantities of peptide. Besides the water-soluble chloramine-T provide a more easily controlled iodination reaction than the reagent iodogen [20]. Based on these facts, we used chloramine-T method for iodination of above mentioned peptide. In previous studies Mamelak *et al.* have used iodogen method to prepare $^{131}$I-chlorotoxin [15].

*In vitro* stability of the rodioiodinated peptide under physiological conditions is an important parameter in the evaluation of *in vivo* dehalogenation [21]. Thus, the stability of the labeled peptide was assessed in the presence of human serum and PBS solution up to 24 h. Results showed that above labeled peptide could resist against dehalogenation in which more than %57 of its structure was remained intact in both PBS and human serum up to 24 h.



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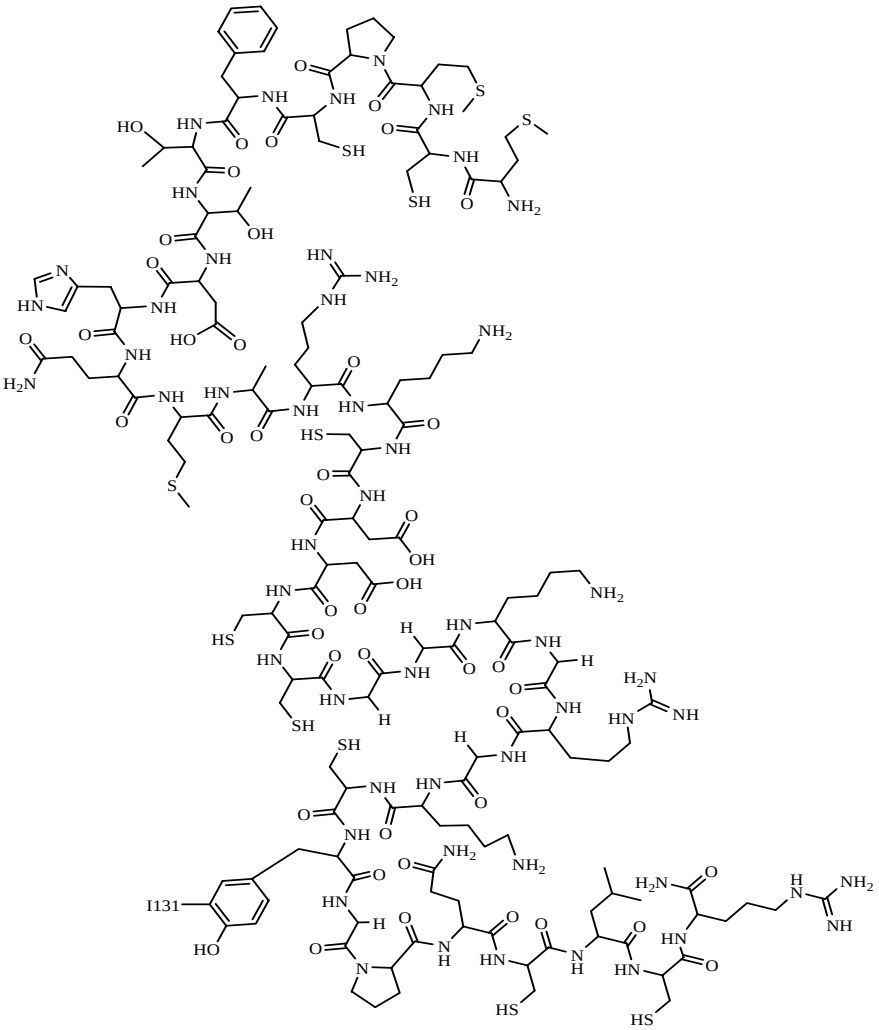

**Figure 1.** Structural formulae of $^{131}$I labeled synthesized chlorotoxin derivative.

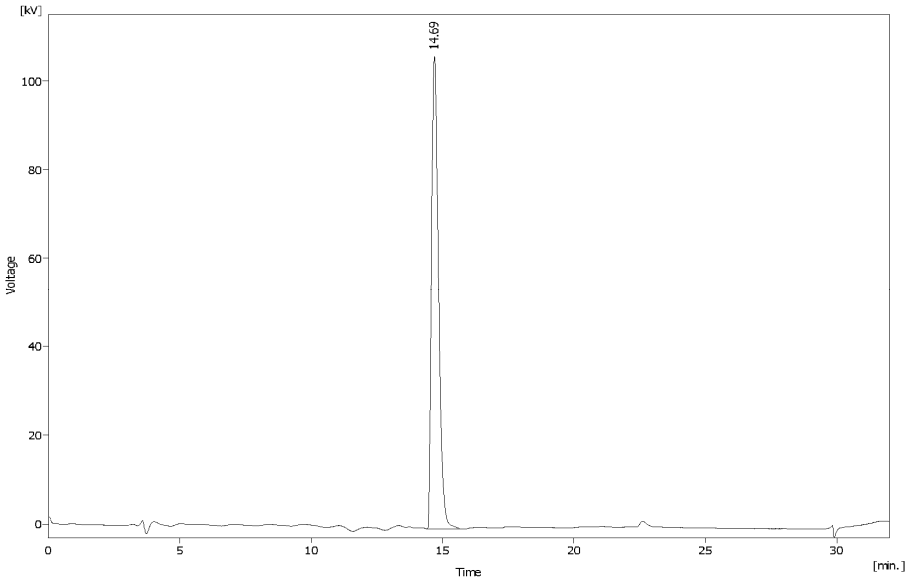


**Figure 2.** RP-HPLC analysis of chlorotoxin derivative.



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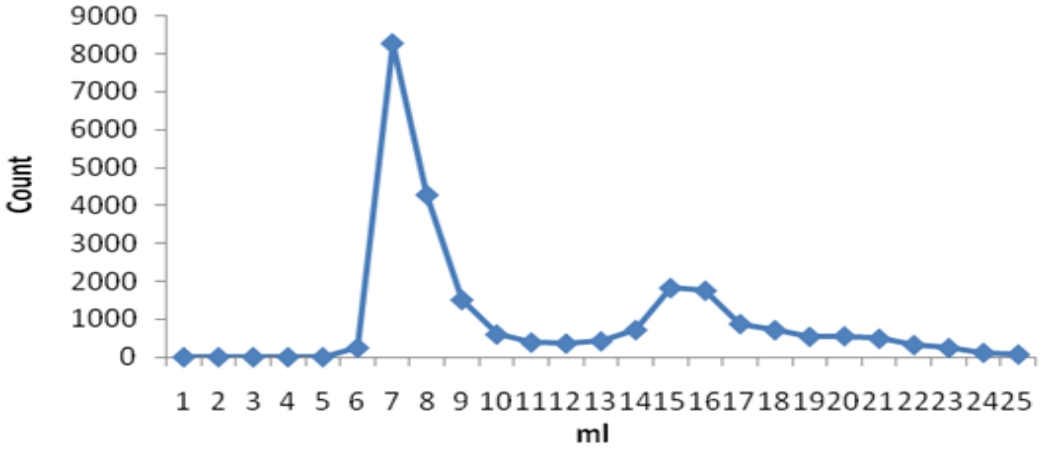


**Figure 3.** Gel filtration chromatography analysis with sephadex G-25 for $^{131}I$-chlorotoxin derivative.

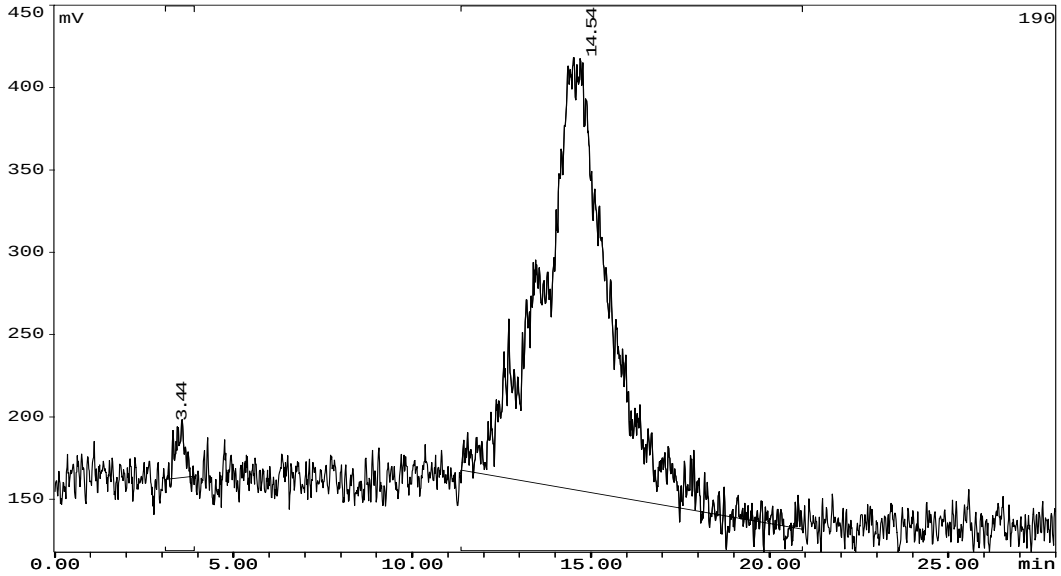


**Figure 4.** RP-HPLC analysis of $^{131}I$-chlorotoxin derivative after purification by sephadex G-25.

The results of biodistribution in mice are summarized in figure 5. Distribution of activity showed that the initial blood level of 0.63% at 1 h decreased to 0.19% after 24 h post injection, which was an indication of the moderate clearance of peptide from the blood circulation system. These Results also showed concentration of activity in kidney and stomach at 1 h post injection. Based on these results one could conclude that the kidneys were the main excretion pathway rather than liver and increase of stomach activity with time can be related with in *in vivo* stability of the compound. Also, brain activity was 0.05 % at 4 h post injection which was an indication that our labeled compound can cross the blood brain barrier.

## CONCLUSION

Our results suggest that, this labeled chlorotoxin derivative might be useful in determining tumor extent and also tumor therapy of gliomas or possibly other cancers.

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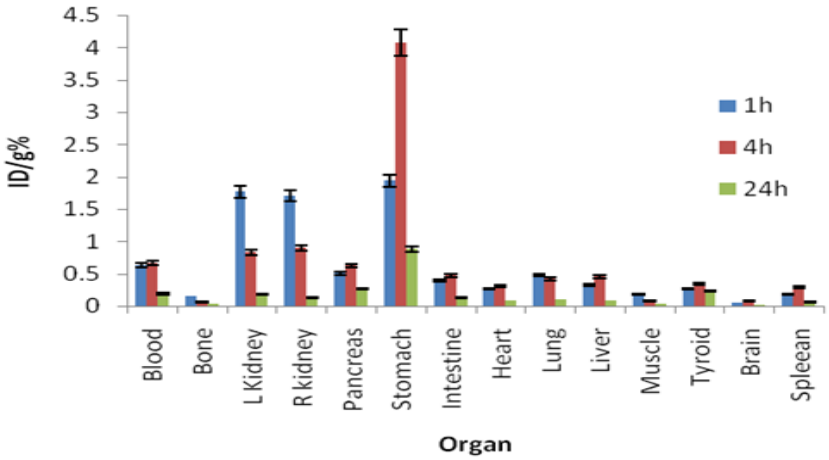


**Figure 5.** Biodistribution of $^{131}$I- chlorotoxin derivative in mouse at time of 1, 4 and 24 h post injection.

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