JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd. Radiopharm 2006; **49**: 951–958
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1115

Research Article

Synthesis and evaluation of ^{90}Y-DOTA-Colchicine conjugate in murine fibrosarcoma model

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Summary

Colchicine is a cytotoxic bioactive alkaloid that exhibits its action by microtubular binding. With an aim to develop a tumor targeted radio-therapeutic agent, colchicine has been functionalized to trimethylcolchicinic acid and conjugated to the isothiocyanato derivative of DOTA (1,4,7,10-tetraaza cyclododecane tetracetic acid). DOTA coupled colchicine was radiolabeled with ^{90}Y, one of the most commonly used therapeutic radioisotope. Complexation of 200 μg of the conjugate with ^{90}Y was carried out at pH 4.5 with an incubation time of 45 min at 70°C. Complexation yield of ^{90}Y-DOTA-NCS-colchicine was confirmed to be >98% using C-18 reverse phase HPLC system. ^{90}Y-colchicine complex could be differentiated from ^{90}Y-*p*-NCS-benzyl-DOTA on the basis of difference in their retention times 8 and 4 min, respectively in a standardized HPLC system. Biodistribution studies in Swiss mice fibrosarcoma tumor model showed an uptake of ~0.8% ID/g tumor at 3 h.p.i. that was retained till 24 h.p.i. ^{90}Y-DOTA-NCS-colchicine complex showed excellent pharmacokinetics with major portion of the radioactivity being excreted out within 3 h.p.i. and no accumulation of radioactivity in vital organs. Copyright © 2006 John Wiley & Sons, Ltd.

Received 28 June 2006; Revised 12 July 2006; Accepted 12 July 2006

Key Words: Colchicine; ^{90}Y; tumor therapy; DOTA

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Introduction

Colchicine is an alkaloid occurring in the seeds of the plant *Colchicum autumnale*. It is one of the natural products used for the treatment of acute gout. Colchicine binds to tubulin and exhibits antimitotic action in dividing cells. The toxic nature of colchicine precludes its use for cancer treatment. Research is being carried out to develop various analogues of colchicine to reduce its toxicity. In this respect, various novel structurally modified colchicine analogues as well as different aspects of mechanism of its biological activity have been reported in recent literature.[1–5] The potential of colchicine for tumor imaging and assessing antiangiogenic effect has been very well documented through ^{99m}Tc-ethylenedicysteine colchicine (^{99m}Tc-EC colchicine).[6] Radiolabeling and evaluation of different colchicine derivatives with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $[^{99m}TcN]^{2+}$ cores for use as tumor imaging agents has already been reported by us.[7] We have tried to explore the potential of colchicine for tumor therapy by radiolabeling it with ^{90}Y, a therapeutic radioisotope.

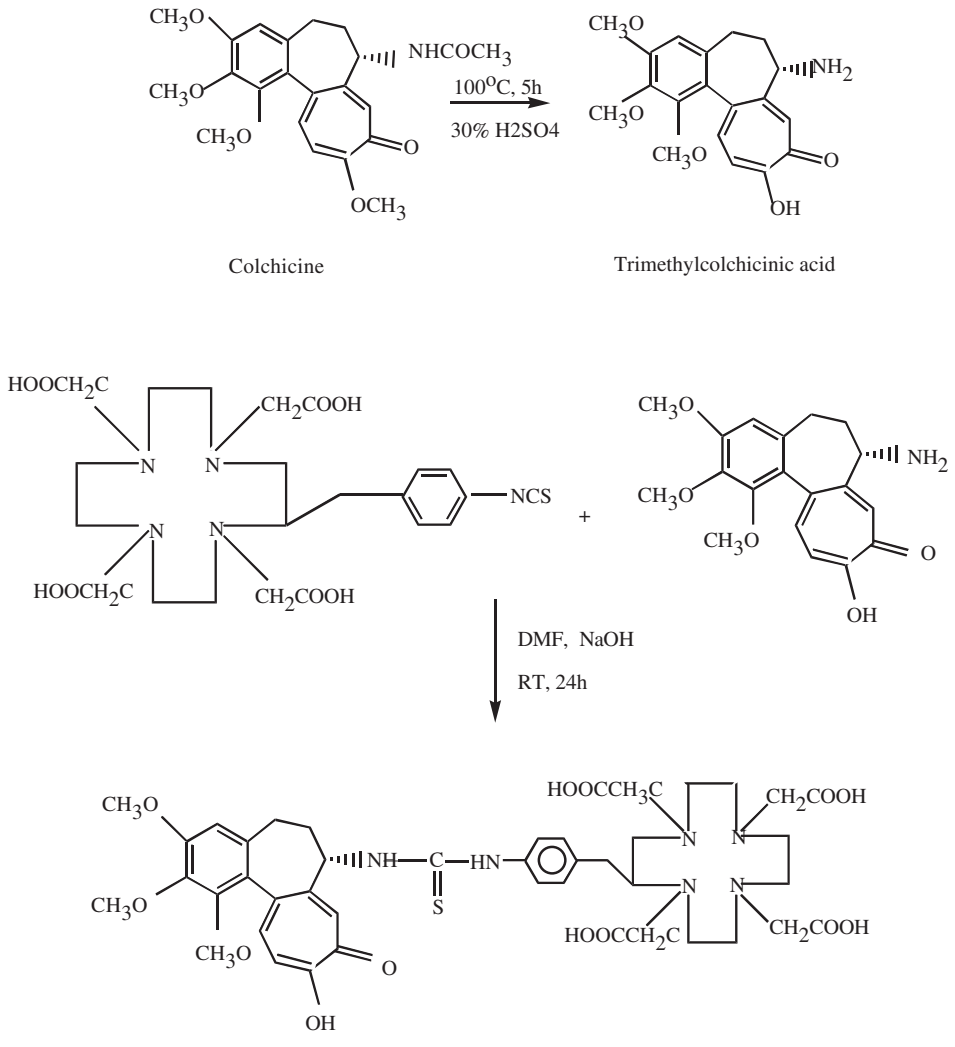
^{90}Y is one of the radioisotopes used for therapy due to its ideal decay characteristics ($T_{1/2} = 64$ h, $E_{\beta-(\max)} = 2.28$ MeV), availability in no-carrier added form from a ^{90}Sr/^{90}Y generator system and amenable chemistry for labeling biomolecules. ^{90}Y, due to its high specific activity has been the preferred choice for radiolabeling of antibodies, peptides, etc. for development of receptor specific therapeutic radiopharmaceuticals.[8,9] The clinical success of the FDA approved radiopharmaceutical Zevalin (^{90}Y-ibritumomab) for treatment of non-Hodgkin's lymphoma has paved the way for ^{90}Y to be a preferred radionuclide for targeted radiotherapy. ^{90}Y is also effective for treatment of solid tumors due to its high range in tissues (11 mm).[10]

As direct labeling of colchicine or its analogues is not easy to achieve with lanthanides, an indirect way of using suitable bifunctional chelating agent (BFCA) is the choice. For labeling with ^{90}Y, colchicine has been modified suitably and then conjugated to a bifunctional chelating agent DOTA-NCS [paraisothiocyanato benzyl 1,4,7,10-tetraazacyclododecane 1,4,7,10 tetra acetic acid (DOTA)] which is known to form kinetically stable complexes with ^{90}Y.[11,12] In this direction, trimethyl colchicinic acid was prepared by acid hydrolysis of colchicine to introduce an amino group which was then further used for conjugation with functionalized DOTA derivative viz. *p*-NCS-benzyl-DOTA. In the present paper, we report studies on preparation of ^{90}Y labeled *p*-NCS-benzyl-DOTA-colchicine and its bio-evaluation in murine fibrosarcoma model.

Results and discussion

Modification of colchicine to a suitable precursor was necessary for subsequent complexation with ^{90}Y. Therefore, acid hydrolysis of colchicine

DOI: 10.1002/jlcr



Scheme 1. Scheme for the synthesis of *p*-NCS-Benzyl-DOTA-colchicine

using 30% concentrated sulphuric acid was carried out to yield trimethyl colchicinic acid.[13] The hydrolysis of the $-NHCOCH_3$ group as well as the concomitant demethylation of $-OCH_3$ group of colchicine could be ascertained by the ^{1}H-NMR. The two 3 H singlets corresponding to $-OCH_3$ and $-NHCOCH_3$ observed in ^{1}H-NMR of colchicine were found to be absent in trimethylcolchicinic acid. Amino group of trimethylcolchicinic acid was then conjugated with BFCA under alkaline conditions to yield the DOTA conjugate of colchicine. (Scheme 1) The formation of the *p*-NCS-benzyl DOTA-colchicine was confirmed by NMR.

The radiolabeling of *p*-NCS-benzyl DOTA-colchicine conjugate with Yttrium-90 was confirmed by HPLC. In the standardized HPLC system

DOI: 10.1002/jlcr

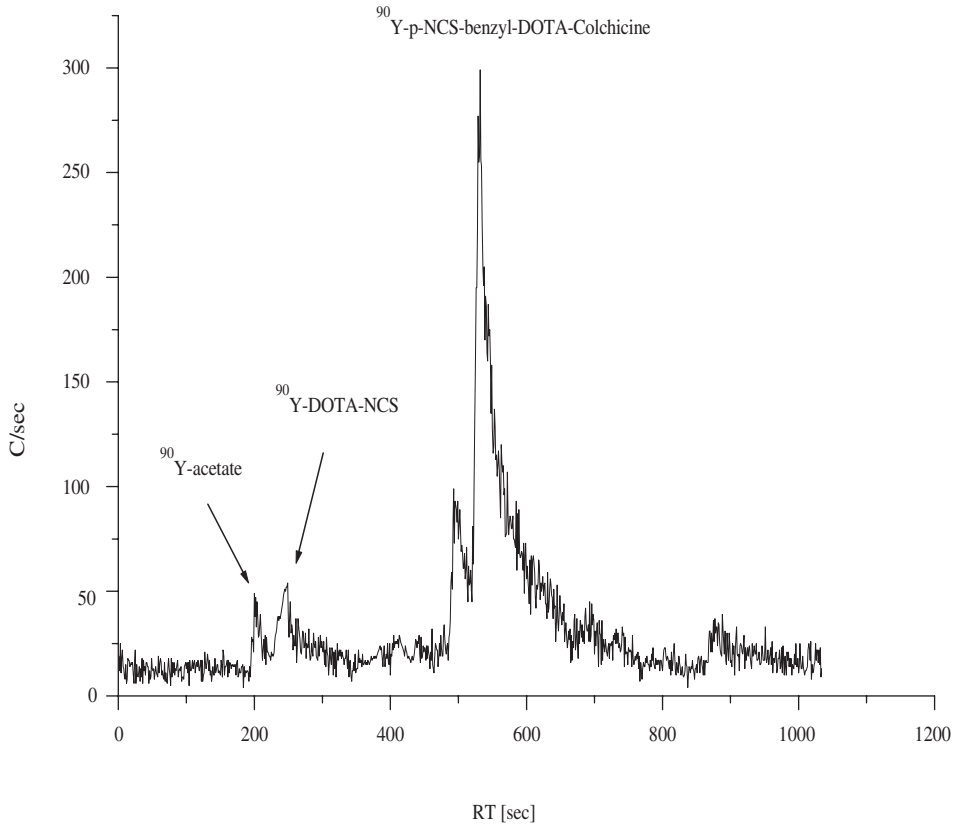


Figure 1. HPLC pattern of ^{90}Y-*p*-NCS-benzyl-DOTA-colchicine

under the conditions of gradient elution, retention time of ^{90}Y-*p*-NCS-benzyl-DOTA was 4 min while that of ^{90}Y-*p*-NCS-benzyl-DOTA-colchicine was 9 min. (Figure 1) The radiolabeling yield was found to vary with the amount of conjugate used. With 100 μg of conjugate ~70% radiolabeling yield was obtained, whereas with 200 μg of the colchicine-DOTA conjugate, >98% radiolabeling could be achieved as determined by HPLC analysis. The radiolabeled product retained >98% radiochemical purity even after 72 h at room temperature as shown by the HPLC analysis. On analysis of the radiolabeled complex by HPLC after 3 h serum incubation, it was found that ~80% activity eluted out at retention time same as that of original complex, indicating ~80% radiochemical purity.

Percentage of injected dose of ^{90}Y-*p*-NCS-benzyl-DOTA colchicine complex per gram of all major organ tissues as well as fibrosarcoma tumor in mice is shown in Table 1. At 3 h.p.i, 0.75 ± 0.15% ID/g tumor uptake was observed which was retained till 24 h.p.i. (0.85 ± 0.35). For *in vivo* application of radiolabeled colchicine, lipophilicity is the major problem as reported in our

DOI: 10.1002/jlcr

Table 1. Biodistribution studies of ^{90}Y DOTA-NCS-colchicine in murine fibrosarcoma model

	% ID/g of tissue	
Organ/Tissue	3 h.p.i.	24 h.p.i.
Blood	0.4 ± 0.07	0.68 ± 0.2
Liver	1.2 ± 0.25	0.3 ± 0.15
Int + GB	3.8 ± 0.68	0.5 ± 0.3
Kidney	8.4 ± 2.0	2.6 ± 0.8
Stomach	2.1 ± 1.03	0.6 ± 0.3
Lungs	0.9 ± 0.25	Nil
Muscle	0.08 ± 0.01	Nil
Femur	Nil	2.4 ± 0.1
Spleen	0.2 ± 0.1	1.5 ± 0.7
Tumor	0.75 ± 0.15	0.85 ± 0.35
T/B	1.9	1.25
T/M	9.4	85

earlier studies of colchicine labeled with different technetium cores.[7] Conjugation with hydrophilic DOTA derivative has resulted in reduced lipophilicity as evident from negligible retention of the complex in the liver. Faster clearance from blood, nearly 90% excretion within 24 h.p.i. and negligible radioactivity in bone led to ~80% increase in target/nontarget ratios with time (T/B, T/M). Even though T/B ratio remained almost same (1.5 at 3 h.p.i. and 1.25 at 24 h.p.i.), T/M ratio was found to increase significantly from 9.3 at 3 h.p.i. to 85 at 24 h.p.i.

Retention of radioactivity in the target organ for longer time is required of a radiopharmaceutical for use as a clinical therapeutic agent. However, most of the radiolabeled small molecules developed and reported for tumor therapy show fast clearance from tumors with negligible retention.[9] ^{90}Y-DOTA-NCS-Colchicine has shown better tumor retention than earlier reported ^{177}Lu labeled sanazole by our group.[14] Recent studies report preferred use of ^{177}Lu along with ^{90}Y for combined therapy of tumors of varied size and stages.[15] Therefore to explore its full potential for tumor therapy, the synthesized colchicine derivative can also be radiolabeled with ^{177}Lu for combined therapy along with ^{90}Y-DOTA-NCS-Colchicine.

Experimental

^{90}Y was obtained from a ^{90}Sr/^{90}Y generator system developed in-house based on a supported liquid membrane technique. *p*-NCS-benzyl DOTA (DOTA-NCS.6HCl) was obtained from M/s. Macrocyclics, USA. All reagents used were AR grade and of high purity. HPLC analyses were performed on a Jasco PU 1580 system with a Jasco 1575 tunable absorption detector and a radiometric detector system. A C-18 reversed phase HiQ Sil (5 μM,

DOI: 10.1002/jlcr

250×4 mm) column has been used. Proton NMR spectra were recorded on 300 MHz Varian VXR 300S spectrophotometer. Fibrosarcoma cell line was procured from 'National Centre of Cell Sciences', Pune, India. Fibrosarcoma tumors were raised in Swiss mice by subcutaneous injection of $\sim 10^6$ cells/animal. All the animal experiments were carried out in compliance with the relevant national laws as approved by the local committee on the conduct and ethics of animal experimentation.

$^{90}Sr/^{90}Y$ *generator*

^{90}Y-chloride for our studies was obtained from a novel ^{90}Sr-^{90}Y generator.[16] In brief, this generator is a cell partitioned into two compartments, namely the feed and the receiver compartment, with a PTFE membrane impregnated with 2-ethyl hexyl-2′-ethyl hexyl phosphonic acid. ^{90}Sr-chloride solution containing ^{90}Sr-^{90}Y at equilibrium (pH 1) in HNO_3 medium is placed in the feed compartment from which $^{90}Y^{3+}$ alone is preferentially transported across the membrane into the receiving chamber filled with 1 M HCl leaving behind the parent radionuclide and other impurities in the feed compartment.

Chemical synthesis

Trimethylcolchicinic acid. Colchicine (150 mg, 0.37 mmol) was dissolved in 4.5 ml of 30% conc. sulphuric acid and heated for 5 h at 100°C in an oil bath. The reaction mixture was neutralized while hot with solid sodium carbonate to pH 7–7.5. The frothy precipitate was filtered and washed with cold water.[13] The filtrate was extracted in chloroform (3×10 ml), the combined extracts were washed with water and dried over anhydrous sodium sulphate. TLC (silica): 5% glacial acetic acid/acetonitrile. R_f (colchicine) = 0.4, R_f (product) = 0. ^{1}H-NMR (CD_3OD, δ ppm) 7.9 (s, 1H, 8-H); 7.42 (d, 11H); 7.16 (d, 12H); 6.7 (s, 1H, 4H) 3.9 (s, 3H, -OCH_3); 3.86 (s, 3H, -OCH_3); 3.6 (s, 3H, -OCH_3); 2.58 (m, 1H, 7H); 2.34 (m, 4H, 5,6H).

Colchicine-DOTA-NCS. Trimethylcolchicinic acid (17 mg, 0.05 mmol) was dissolved in 2 ml DMF and *p*-NCS-benzyl DOTA (18 mg, 25 μmol) was added to it along with NaOH (1 mg, 0.025 mmol) to maintain alkaline conditions. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed under vacuum and ~20 mg of light yellow product was obtained. ^{1}H-NMR (D_2O, δ ppm) 8.3 (s, 1H, 8H); 7.16 (m, 4H, PhDOTA); 6.97 (d,1H, 11H); 6.93 (d, 1H, 12H); 6.7 (s, 1H, 4H); 3.8 (s, 8H, -CH_2COOH); 3.78 (s, 3H, -OCH_3); 3.76 (s, 3H, -OCH_3); 3.74 (s, 3H, -OCH_3); 3.43 (t, 1H, 7H); 3.22 (s, 2H, -CH_2Ph); 2.68 (m, 1H, -$NCHCH_2N$-); 2.64 (m, 4H, 5,6H); 2.2 (m, 14H, -NCH_2CH_2N-).

DOI: 10.1002/jlcr

Radiolabeling

$^{90}YCl_3$ was converted to ^{90}Y acetate by mixing $^{90}YCl_3$ (74 MBq) and 0.5 M ammonium acetate (1:1 v/v). The pH of ^{90}Y acetate was adjusted to 4–5 with 3 N NaOH. Radiolabeling was carried out with varying amounts of conjugate. In a typical protocol, DOTA-NCS-Colchicine conjugate (200 μg, 0.2 μmol) was added to ^{90}Y acetate (37 MBq) and the reaction mixture was incubated at 70°C for 45 min.

Under similar conditions, ^{90}Y-DOTA-NCS was prepared using 50 μg of DOTA-NCS and 37 MBq of ^{90}Y acetate.

Characterization

^{90}Y-DOTA-NCS-Colchicine as well as ^{90}Y-DOTA-NCS were characterized by HPLC. About 25 μl of the radiolabeled complex was injected into the column (C18 reverse phase) where H_2O (solvent A) and acetonitrile (solvent B) with 0.1% trifluoroacetic acid were used for elution in the gradient system: 0–28 min, 90%A–10%A; 28–30 min, 10%A; 30–32 min, 10%A–90%A. The flow rate was maintained at 1 ml/min and the elution was monitored by the radioactivity profile.

Stability studies

In vitro serum stability was studied by incubating the radiolabeled complex in serum at 37°C for 3 h. To determine the radiochemical purity after serum incubation, the product was extracted in acetonitrile and characterized by HPLC.

Biodistribution studies

Biodistribution studies were carried out in fibrosarcoma bearing Swiss mice. 0.1 ml of the product containing 3.7 MBq of activity was injected via tail vein. The studies were carried out in triplicate at 3 h and 24 h post-injection. All major organs as well as tumor were excised, weighed and counted in a NaI (Tl) flat geometry detector in order to estimate the percent of injected dose per gm of the tissue. Blood, muscle and bone were taken as 7, 40 and 10% of total body weight. All the animal studies were carried out in compliance with national laws as approved by local animal ethics committee.

Conclusion

Trimethyl colchicinic acid was conjugated to the bifunctional chelating agent, namely DOTA and this conjugate could be successfully radiolabeled with ^{90}Y in high yields. The most promising feature of the product was retention of radioactivity in fibrosarcoma tumor even after 24 h with fast clearance from other vital organs. The results lead to a conclusion that colchicine conjugate

DOI: 10.1002/jlcr

labeled with Yttrium-90 may have a potential for treatment of large size solid tumors due to high beta energy of the radioisotope.

Acknowledgements

Authors are grateful to Dr H. D. Sarma for his support to carry out the biodistribution studies. We also express gratitude for Dr V. Venugopal, Director Radiochemistry and Isotope Group, Bhabha Atomic Research Centre, for his constant support and encouragement.

References

1. Hello C Le. In *The Alkaloids*, vol. 53, Cordell GA (ed.). Academic Press: San Diego, 2000; 288–352.
2. Nogales E, Wolf SG, Downing KH. *Nature* 1998; **391**: 199–203.
3. Zhang SX, Feng J, Kuo SC, Brossi A, Hamel E, Tropsha A, Lee KH. *J Med Chem* 2000; **43**: 167–176.
4. Hendrikse NH, Franssen EJF, Van der Graaf WTA, Vaalburg W, de vries EGE. *Eur J Nucl Med* 1999; **26**: 283–293.
5. Levchenko A, Mehta BM, Lee JB, Humm JL, Augensen F, Squire O, Kothari PJ, Finn RD, Leonard EF, Larson SM. *J Nucl Med* 2000; **41**: 493–501.
6. Zareneyrizi JF, Yang DJ, Oh CS, Ilgan S, Yu DF, Tansey W, Liu CW, Kim EE, Podoloff DA. *Anticancer Drugs* 1999; **10**: 685–692.
7. Korde A, Satpati D, Mathur A, Mallia M, Banerjee S, Kothari K, Sarma HD, Choudhari P, Venkatesh M. *Bioorg Med Chem* 2006; **14**: 793–799.
8. Venkatesh M, Pandey U, Dhami PS, Kannan R, Achuthan PV, Banerjee S, Samuel G, Pillai MRA, Ramanujam A. *Radiochim Acta* 2001; **89**: 413–417.
9. Volkert WA, Hoffman TJ. *Chem Rev* 1999; **99**: 2269–2292.
10. Grillo-Lopez AJ. *Expert Rev Anticancer Ther* 2002; **2**: 485–493.
11. Kodama M, Koike T, Mahatma AB, Kimara E. *Inorg Chem* 1991; **31**: 1270–1273.
12. Stimme JB, Kull Jr FC. *Nucl Med Biol* 1998; **25**: 117–125.
13. Kothari JP, Finn DR, Larson MS. *J Label Compd Radiopharm* 1994; **36**: 521–528.
14. Das T, Chakraborty S, Banerjee S, Samuel G, Mukherjee A, Sarma HD, Venkatesh M. *Bioorg Med Chem* 2004; **12**: 6077–6084.
15. De Jong M, Breeman WAP, Valkema R, Bernard BF, Krenning EP. *J Nucl Med* 2005; **46**: 13S–17S.
16. Achuthan PV, Dhami PS, Kannan R, Gopalakrishnan V, Ramanujam A. *Separation Sci Technol* 2000; **35**: 261–270.

DOI: 10.1002/jlcr