IJPT
0000-0000/02/12-24-29
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS
Copyright © 2003 by Razi Institute for Drug Research (RIDR)
IJPT 1:24-29, 2003

Dynamic Distribution of ^{67}Ga-Bleomycin Complex and Carrier Free ^{67}Gallium in Normal Mice

FARAJ TABEIE, BAHRAM BOLOURI, AMIR REZA JALILIAN, NARIMAN MOSSAFFA, HOSEIN RAJABI, EISA NESHANDAR ASLI, FARZANEH LABIBI, ALI REZA KARIMIAN and BEHROOZ SHIRAZI

Department of Medical Physics & Engineering, Shaheed Beheshti University of Medical Sciences (F.T.); Department of Biophysics & Medical Physics, Iran University of Medical Sciences (B.B.); Cyclotron & Nuclear Medicine Department, Nuclear Research Center for Agriculture & Medicine (NRCAM), Atomic Energy Organization of Iran (AEOI) (A.R.J., A.R.K., B.S.); Department of Immunology, Shaheed Beheshti University of Medical Sciences (N.M.), Department of Medical Physics, Tarbiat Modarres University, College of Medical Basic Sciences (H.R.); Department of Nuclear Medicine, Shaheed Beheshti University of Medical Sciences (E.N.A.); Department of Pharmacology, Shaheed Beheshti University of Medical Sciences (F.L.)

Received September 28, 2002; Revised January 12, 2003; Accepted February 3, 2003

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

This study reports the labeling of Gallium-Bleomycin (^{67}Ga-BLM) complex as a radiopharmaceutical and optimization of its labeling conditions; pH, reaction time, temperature, concentration of bleomycin and its biodistribution in normal Bulb C mice. The biodistribution of the complex was compared with ^{67}Ga-Cl_3 in 11 selected organs including blood, liver, lung, spleen, muscle, skin, heart, kidney, colon, colon content, and bladder at five selected times of 1, 2, 4, 24 and 48 hours after injection. Cyclotron produced ^{67}Gallium was labeled with bleomycin under Thakur method. The optimized pH condition was found 2 at temperature of 90ºC for reaction temperature of 30 minutes when 0.5 mg of bleomycin was mixed with 1 mCi of ^{67}Ga-Cl_3. Pharmacokinetic data indicated higher uptakes of ^{67}Ga-BLM in all 11 tissues except blood, liver and spleen in comparison with ^{67}Ga-Cl_3. The average of total uptakes from ^{67}Ga-BLM and ^{67}Ga-Cl_3 radiopharmaceuticals at one hour after injection were 73.35% and 53.55% then reduced to 14.55% and 25.2% after 48 hours respectively. The blood uptake of ^{67}Ga-Cl_3 was higher than ^{67}Ga-BLM in all time intervals. Bladder uptake of ^{67}Ga-BLM was highest among 11 tissues at all time intervals but the uptake of ^{67}Ga-Cl_3 was only highest at first hour after the injection. The results indicated the high stability of the complex both in-vitro and in-vivo, and yet excreted faster than carrier free ^{67}Gallium. The effective half life of ^{67}Ga-BLM complex was found 48.15 hours.

Keywords: *Radiopharmaceutical, ^{67}Gallium-Bleomycin, Labeling, Biodistribution*

Bleomycins (BLMs) are group of glycopeptides antitumor antibiotics that were first obtained by Umezawa from cultures of Streptomyces verticillus in 1962 [1]. Bleomycin (BLM) contains 13 peptide in two main groups A (A1-A7) and B (B1-B6), of those A2 and B2 are of the major effective forms. Bleomycin has significant antitumor activity against several human malignancies including squamous cell carcinoma, malignant lymphomas, germ cell tumors of testes [2, 3], Kaposi`s sarcoma, malignant melanoma [4, 5], head and neck cancers [6].The mechanisms of BLM induced cytotoxicity have been studied [7, 8] and when in presence of Fe^{+2} ion and oxygen, it transforms into its active form and binds to DNA molecules. Reaction of active form of BLM with DNA backbone generates C4 radical carbon on deoxyribose which finally leads to DNA single strand breaks (SSB) and/or double strand breaks (DSB) and cell death [9-11]. In efforts to expand the use of BLM, studies have been carried out to investigate the cell killing effects of combined treatment with different physical conditions i.e. use of intense electric pulse [12-18] and hyperthermia [19, 20].

During the past three decades, complexes of bleomycin with radioactive elements as a radiopharmaceutical and their body distribution and resultant biological effects, were intensively studied. This interest had different reasons. First, by labeling BLM with gamma emitter radioactive elements, there would be a potential to study spatial and temporal biodistribution of the complex in the body through nuclear medicine imaging protocols [21-23]. Second, if labeling of BLM were performed by Beta or Auger emitter radiotracers, there would be ability of studying combined effects of ioniz-

ing radiation as well as chemotherapy on different organs bearing tumors [23-27].

The BLMs can chelate a wide variety of two and three valent metal ions including Fe^{+2}, Co^{+2}, Mn^{+2}, In^{+3} and Ga^{+3} [21-27]. Several radioactive complexes of BLM has been used by different investigators including Indium-111, Cobalt-57, Technetium-99m and Iodine-131 [21-26]. Among the Radiolabels of BLM the Indium-111 BLM complex was widely studied in the past three decades. Thakur introduced labeling of BLM with Indium-111 in 1973 and mentioned the possibility of labeling BLM with other three valent radiolabels like Gallium-67 [28]. Biodistribution and stability of Indium-111 BLM complex were studied in normal and tumoral animal models by many investigators [21-27, 29].

Gallium-67 with 78 hours physical half-life has high tumor affinity and it is being used in nuclear medicine for detection and imaging of malignant and inflammatory sites in the body for a long time. Yet among all the radiolabels of BLM, not much has been reported on Gallium-Bleomycin complex. Hence this study concerns the preparation of this radiopharmaceutical complex, its quality control, optimization of labeling conditions, stability and biodistribution in normal animal model. Further to this the follow up study will cover the distribution of the complex in tumoral model and its employment in enhancing the therapeutic effects via electroporation method.

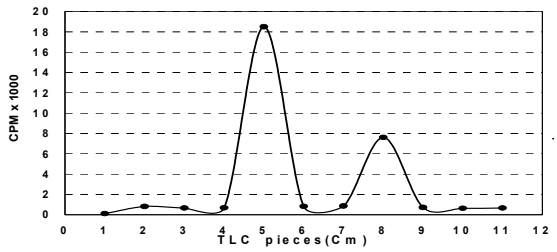

Fig 1. Radiochromatograph of ^{67}Ga-BLM at optimized condition

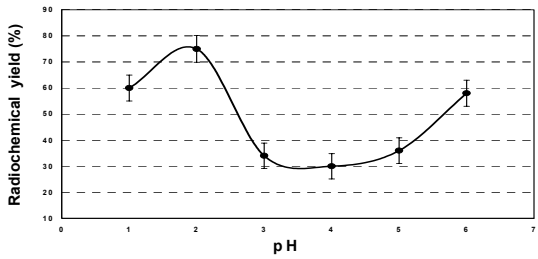

Fig 2. Effect of pH on radiochemical yield of ^{67}Ga-BLM

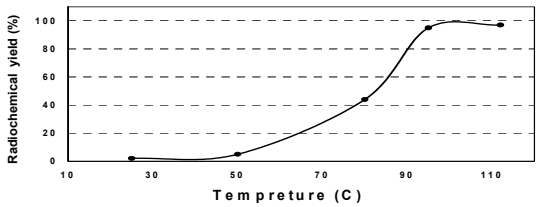

Fig 3. Effect of tempreture on radiochemical yield of ^{67}Ga-BLM

MATHERIALS AND METHODS

Labeling. Gallium-67 is commonly used in nuclear medicine because of its favorable decay characteristics. This radioisotope with 78 hours of physical half life emits low energy Auger electrons and photons of 93, 184 and 296 kilo electron Volts. Gallium-67 was prepared by 28 Million electron Volts proton bombardment of enriched zinc target in cyclotron (Cyclon-30, IBA). Irradiated target dissolved in HCl 10 N and passed through cation exchange and then washed by HCl 4N solution. Finally Ga-67 in form of ^{67}GaCl 3 was used for labeling of Bleomycin.

Gallium chloride solution in 2 ml vial with 0.25-2.5 mCi activity was adjusted to pH 1 by HCl 1 M and/or NaOH 1 M. The vial solution was evaporated by slight heating under nitrogen gas flow. The Gallium-67 residue was mixed with 0.25-2.5 mg bleomycin in 0.1 ml normal saline. The mixture reaction performed at different temperatures (25, 50, 80 and 100°C) and samples of yielding complex were taken in each temperature with 30 minutes intervals for quality control.

Radiochemical purity tests were carried out by Radio Thin Layer Chromatography (RTLC) on polymer backed silica gel papers. The mobile phase of chromatography was 10% ammonium acetate and methanol with equal volumes. Silica gel papers sized 2×10 cm containing samples was dried by N2 gas and counted by

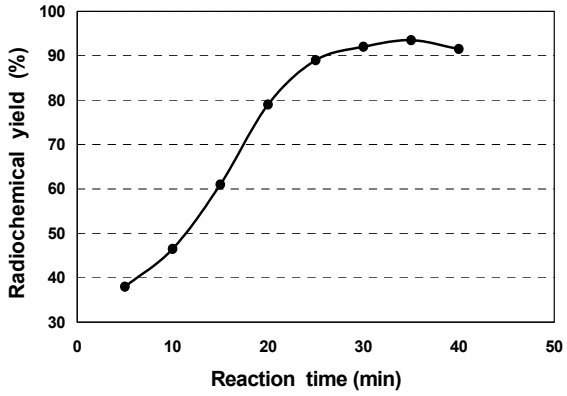

Fig 4. Effect of reaction time on radiochemical yield of ^{67}Ga-BLM

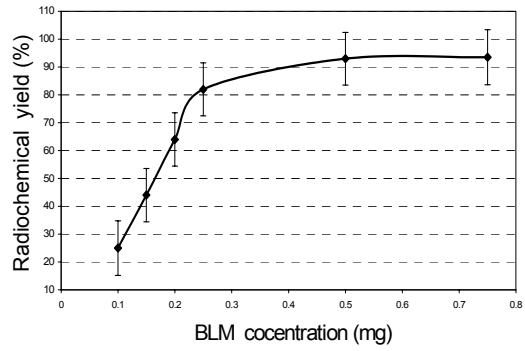

Fig 5. Effect of bleomycin concentration on radiochemical yield of ^{67}Ga-BLM

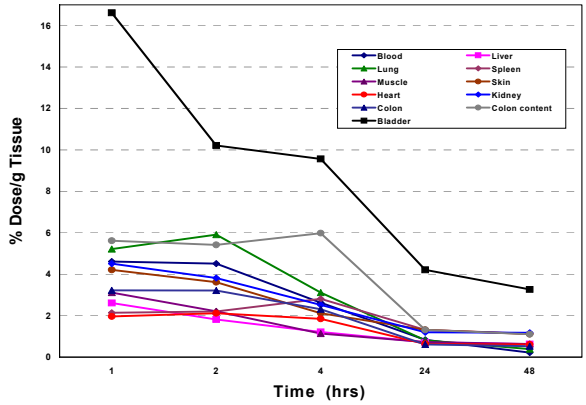

Fig 6. Distribution of ^{67}Ga-BLM in normal mice

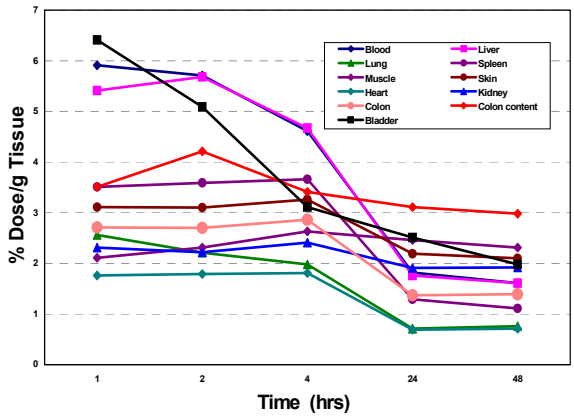

Fig 7. Distribution of ^{67}Ga-Cl_3 in normal mice

gamma spectroscope with HPGE detector in 0.5 cm steps for 10 minutes in each step. The radiochemical yields were also determined by KPLC (Waters-C18 reverse phase radial pack) and ITLC (Whatman-SG/MEK). The above procedure was repeated for other pH values including 2-7 for labeling pH optimization. Finally the optimum pH [2] and temperature (100°C), and labeling time (1.5 hours) for the production of the complex were used in this study.

Stability of complex. 50 µL samples of the complex were taken at 2, 4, 8, 12 and 24 hours intervals after labeling period and their in-vitro stability were checked by RTLC method. This test was repeated in presence of human and mice serum as well. Then the in-vivo stability for the complex was evaluated when conducting the biodistribution of complex.

Biodistribution. Biodistribution and in vivo stability of complex studies was performed on 48 female Bulb C mice of 20 g weight each. Twenty four mice of group of 6, received 60 µCi of ^{67}Ga-BLM complex intravenously via dorsal tail vein. Five groups of animals were killed 1, 2, 4, 24 and 48 hours after injection of the radiopharmaceutical. Samples of 11 organs including blood, liver, lung, heart, spleen, muscle, skin, kidney, colon, colon content and bladder were excised, weighed wet and counted by NaI (Tl) well counter (Capintek 2). Extra care and cautious were observed while performing the excision and the activity counts of the tissue samples. The absolute tissue concentrations expressed as a percentage of the administered dose per gram of the wet tissue. The sixth group was undergone dynamic imaging by dual head gamma camera with high energy parallel hole collimator concurrently with the time intervals of tissue excisions of the other five groups post-injection. When imaging, to limit the animal motion, using no anesthetics, a specially designed plexiglass restrainer was employed. The above procedure was repeated on other 24 mice by administration of ^{67}Ga-Cl_3 as a carrier free radiopharmaceutical.

RESULTS

Labeling. Radio-thin layer chromatography of produced complex, showed two distinct radiopeaks with Rf of 0.40 and 0.70 as indicated in Fig 1. In all radiolabeling procedures (n=50), the radioactivity ratio of two peaks were constant (0.4, 0.7), showing isometric

Table 1. Distribution of ^{67}Ga-BLM and ^{67}Ga-Cl_3 in Normal mice

Time (hrs)		1		2		4		24		48	
Organ		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood	Ga-BLM	4.61	0.96	4.51	1.15	2.62	0.56	0.83	0.13	0.21	0.05
	Ga-Cl3	5.91	1.12	5.71	1.22	4.61	0.16	1.82	0.17	1.61	0.1
Liver	Ga-BLM	2.61	0.36	1.81	0.45	1.21	0.47	0.71	0.14	0.62	0.08
	Ga-Cl3	5.41	1.06	5.68	1.23	4.67	0.86	1.76	0.34	1.61	0.42
Lung	Ga-BLM	5.21	1.4	5.91	1.11	3.11	0.85	0.81	0.25	0.38	0.15
	Ga-Cl3	2.56	0.38	2.21	0.68	1.98	0.36	0.71	0.14	0.76	0.12
Spleen	Ga-BLM	2.14	0.63	2.2	0.56	2.81	0.68	1.31	0.16	1.12	0.21
	Ga-Cl3	3.51	0.82	3.59	0.41	3.66	0.84	1.29	0.68	1.11	0.35
Muscle	Ga-BLM	3.11	0.51	2.21	0.54	1.13	0.32	0.72	0.13	0.63	0.15
	Ga-Cl3	2.11	0.21	2.31	0.59	2.63	0.46	2.46	0.14	2.31	0.13
Skin	Ga-BLM	4.21	0.78	3.61	0.54	2.11	0.31	1.31	0.18	1.11	0.12
	Ga-Cl3	3.11	0.41	3.1	0.89	3.26	0.24	2.19	0.14	2.1	0.11
Heart	Ga-BLM	1.96	0.31	2.11	0.39	1.84	0.51	0.63	0.24	0.61	0.18
	Ga-Cl3	1.76	0.25	1.79	0.47	1.81	0.41	0.69	0.24	0.71	0.15
Kidney	Ga-BLM	4.51	0.85	3.81	0.75	2.51	0.71	1.19	0.38	1.17	0.32
	Ga-Cl3	2.31	0.54	2.22	0.55	2.41	0.67	1.91	0.35	1.92	0.24
Colon	Ga-BLM	3.22	0.36	3.21	0.41	2.31	0.51	0.61	0.19	0.52	0.18
	Ga-Cl3	2.71	0.49	2.7	0.61	2.86	0.71	1.37	0.49	1.39	0.28
Colon content	Ga-BLM	5.61	0.96	5.41	0.85	5.98	1.16	1.31	0.32	1.1	0.24
	Ga-Cl3	3.51	0.76	4.21	0.97	3.41	0.78	3.11	0.37	2.98	0.26
Bladder	Ga-BLM	16.61	2.98	10.21	2.31	9.56	2.39	4.21	0.44	3.26	0.19
	Ga-Cl3	6.41	1.44	5.09	1.14	3.11	0.74	2.51	0.35	1.98	0.13
Average	Ga-BLM	4.89		4.09		3.19		1.24		0.97	
	Ga-Cl3	3.57		3.51		3.12		1.8		1.68	

ratio of the three bleomycin components corresponding to bleomycin A2, B2 and bleomycinic acid. A mean molecular weight of 1495 was calculated using the above ratios, in order to obtain the approximate specific activity of 1.39 Ci/mmole. In order to obtain the best labeling conditions, the complex formation was optimized for pH, temperature, reaction time and the concentration of bleomycin. At an arbitrary temperature (80ºC), the best pH for labeling was found 2 as indicated in Fig 2. Increasing the temperature of complex mixture during reaction time to 90ºC increased the yield, which remained constant for temperatures up to 100ºC (Fig 3). The radiopharmaceutical yield above 90ºC temperature reduced due to possible bleomycin decomposition. At the optimum reaction temperature and pH, the yield reached a maximum within 25-30 minutes and remained constant for longer reaction times (Fig 4). Increasing the concentration of bleomycin relative to ^{67}Ga-Cl_3 (1 mCi) increased the yield of complex reaching to a saturation value of about 0.5 mg as shown in Fig 5.

Biodistribution. Tissue pharmacokinetic data are reported in Table 1. For each tissue listed in the table, the mean percent of initial administered activity per unit weight of the tissue of the samples (n=4 mice) for both radiopharmaceuticals ^{67}Ga-BLM and ^{67}Ga-Cl_3 at five selected time intervals were calculated. In addition the in all 11 organs except blood, liver, and spleen were higher than ^{67}Ga-Cl_3.

The average of percent dose per unit weight of selected tissues from ^{67}Ga-BLM at 1 hour after injection was .89% which reduced to 0.97% after 48 hours. Hence the average of total uptake from ^{67}Ga-BLM at 1 hour after injection was 73.35% then reduced to 14.55% after 48 hours. The average of percent dose per unit weight of selected tissues from ^{67}Ga-Cl_3 at one hour after injection was 3.57% which reduced to 1.68% after 48 hours. Thus the average of total uptake from ^{67}Ga-Cl_3 at one hour after injection was 53.55% then reducing to 25.2% after 48 hours. This implies that clearance of ^{67}Ga-BLM is more than two times faster than ^{67}Ga-Cl_3 after 48 hours of administration.

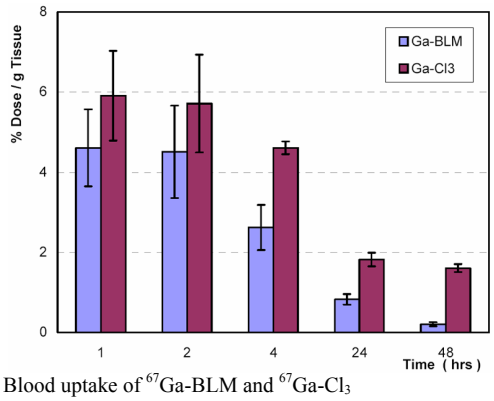

Fig 8. Blood uptake of ^{67}Ga-BLM and ^{67}Ga-Cl_3

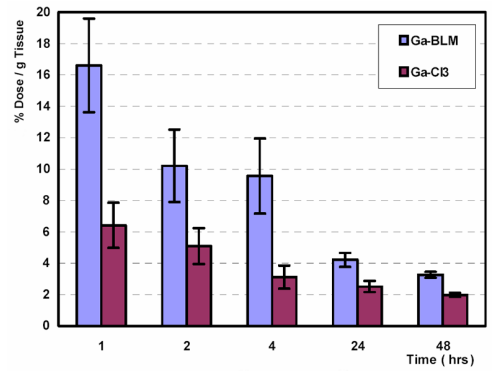

Fig 9. Bladder uptake of ^{67}Ga-BLM and ^{67}Ga-Cl_3

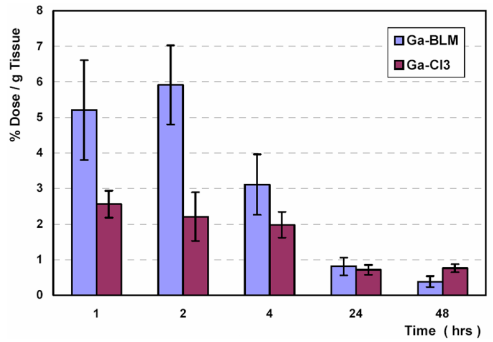

Fig 10. Lung uptake of ^{67}Ga-BLM and ^{67}Ga-Cl_3

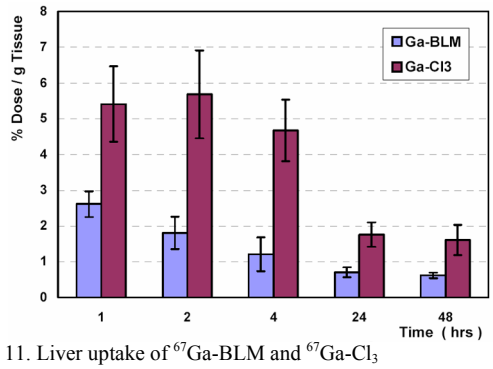

Fig 11. Liver uptake of ^{67}Ga-BLM and ^{67}Ga-Cl_3

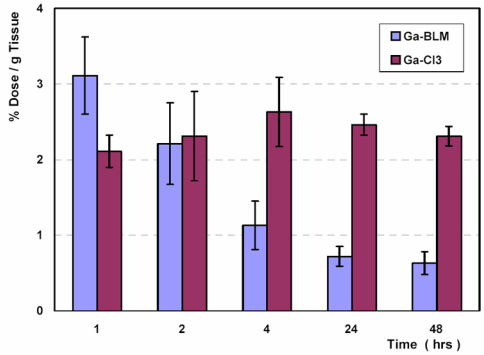

Fig 12. Muscle uptake of ^{67}Ga-BLM and ^{67}Ga-Cl_3

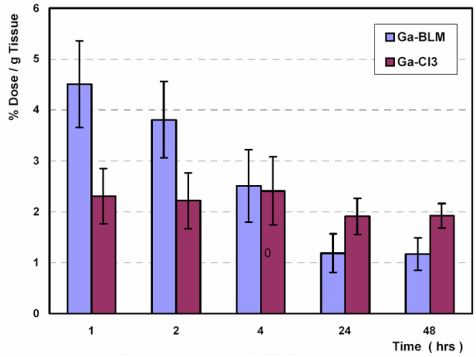

Fig 13. Kidney uptake of ^{67}Ga-BLM and ^{67}Ga-Cl_3

The blood uptake (Fig 8) of ^{67}Ga-Cl_3 was higher than ^{67}Ga-BLM in all time intervals, but blood clearance of ^{67}Ga-BLM after 48 hours (95%) was faster than ^{67}Ga-Cl_3 (72%). Bladder uptake of ^{67}Ga-BLM was highest among 11 tissues at all selected times after injection (Fig 6) but ^{67}Ga-Cl_3 uptake was highest at first hour (Fig 7). Bladder uptakes of both radiopharmaceuticals are compared in Fig 9.

Bladder excretion of ^{67}Ga-BLM in all time intervals was more than two times faster than ^{67}Ga-Cl_3. The Lungs uptake for two radiopharmaceuticals was compared as in Fig 10 indicating faster clearance for ^{67}Ga-BLM (92%) in comparison with ^{67}Ga-Cl_3 (70%). In contrast to the lungs, the clearance of liver for both radiopharmaceuticals are almost the same (76% and 70%) as showing in Fig 11. The other seven tissue uptakes are summarized in Fig 6 and 7. Muscle and kidney uptakes are also shown in Fig 12 and 13 indicating similar uptake patterns. The uptake and the clearance of ^{67}Ga-BLM in both tissues showed two distinct increasing and decreasing phases, however for ^{67}Ga-Cl_3 were decreasing almost with the same clearance rate.

Discussion and Conclusion

This study presents labeling of a chemotherapeutic drug, bleomycin, with ^{67}Gallium radiolabel under optimized condition including: pH, reaction time, reaction temperature, bleomycin concentration and quantification of its distribution in 11 selected normal tissues. The optimum pH of 2 is in full agreement with previous investigator's reports that used low pH values in the range of 1-2 [21-25]. The optimum reaction time of 25 minutes found in this study for the complex agrees with Thakur method for labeling of ^{111}In-Bleomycin complex [28]. Yet some investigators have reported higher reaction times of up to one hour [23, 25]. ^{67}Ga-BLM complex showed mean uptake of 73.5% at one hour after injection which then reduced to 14.55% at 48 hours by a clearance of 80%. The time required for fifty percent clearance "effective half life" estimated to be 48.15 hours. ^{67}Ga-BLM in comparison with ^{67}Ga-Cl_3, had 1.37 times higher uptake at one hour after injection and 1.53 times clearance rate. The ^{111}In-BLM complex in comparison with ^{67}Ga-BLM has lower uptake in one hour, but with the same clearance rate [29]. On the other hand, ^{111}In-Cl_3 has about 92% uptake at one hour post injection with a clearance of 42%. This shows a faster uptake but lower clearance. In conclusion the prepared complex rendered high stability in-vivo and high clearance in normal tissues. Investigations on the application of the complex for various tumoral models for therapeutic purposes are suggested (a different report is being submitted elsewhere).

Acknowledgments

The authors wish to thank the following at the Nuclear Research Center for Agriculture & Medicine (NRCAM), Atomic Energy Organization of Iran (AEOI), Karaj, Iran: Dr Gh.R. Raeisali, Dr H. Rafiei and the colleagues in the department of Cyclotron and Nuclear Medicine: Seddighe Moradkhani and Rozitta Dejbankhan for their support and help along the animal experimentation, radiopharmaceutical preparation and nuclear medicine imaging.

References

1. Umezawa H, Maeda K, Takeuchi T, Okami Y. New antibiotics bleomycin A and B. *J Antibiot* 1996;**19:**200-209.
2. Blum RH, Carter SK, Agre K. Clinical review of bleomycin – a new antineoplastic agent. *Cancer* 1973;**31:**903-914.
3. Crooke ST, Bradner WT. Bleomycin, a review. *J Med* 1976;**7:**333-428.
4. Shepherd FA, Mayer E, Cardella C, Cole E, Greig P, Wade JA, et al. Treatment of caposi`s sarcoma after solid organ transplantation. *J Clin Oncol* 1997;**15:**2371-2377.
5. Glass LF, Pepine ML, Fenske NA, Jaroszeski M, Reintgen DS, Heller R. Bleomycin-mediated electro-chemotherapy of metastatic melanoma. *Arch Dermatol* 1996;**132:**1353-1357.
6. Hofmann GA, Dev SB, Dimmer S, Nanda GS. Electroporation therapy: A new approach for the treatment of head and neck cancer. *IEEE Trans Biomed Eng* 1999;**46:**752-759.
7. Umezawa H, Ishizuka M, Maeda K, Takeuchi T. Studies on bleomycin. *Cancer* 1967;**20:**891-895.
8. Hecht SM. Bleomycin: new perspectives on the mechanism of action. *J Nat Prod* 2000;**63:**158-168.
9. Suzuki H, Nagai K, Yamaki H, Tanaka N, Umezawa H. On the mechanism of action of bleomycin: Scission of DNA strsnds in vivo and in vitro. *J Antibiot (Tokyo)* 1969;**22:**446-448.
10. Terasima T, Yasukawa M, Umwzawa H. Breaks and rejoining of DNA in cultured mamalian cells treated with bleomycin. *Jpn J Cancer Res* 1970;**61:**513-516.
11. Yamaki H, Suzuki H, Nagai K, Tanaka N, Umwzawa H. Effects of bleomycine A2 on deoxy-ribonuclease, DNA polymerase and ligase reactions. *J Antibiot (Tokyo)* 1971;**24:**178-184.
12. Hyacinte M, Jariszeski MJ, Dang V, Coppola D, Karl RC, Gilbert RA, et al. Electrically enhanced drug delivery for the treatment of soft tissue sarcoma. *Cancer* 1999;**75:**409-417.
13. Mir LM, Orlowski S, Belehradek J, Paoletti A. Potentiation of antitumor effects of bleomycin by local electric pulses. *Eur J Cancer* 1991;**27:**65-72.
14. Heller R, Jaroszeski M, Perrott R, Messina J, Gilbert R. Effective treatment of B16 melanoma by direct delivery of bleomycin using electrochemotherapy. *Melanoma Res* 1997;**7:**170-180.
15. Rudolf Z, Stabuc B, Cemazar M, Micalavic D, Vodovnic L, Sersa G. The first clinical experience in malignant melanoma patients. *Radiol Oncol* 1995;**29:**229-235.
16. Domenge C, Orlowski S, Luboinski B, De Baere T, Schwabb G, Belehradek J, et al. Antitumor electrochemotherapy: New advances in clinical protocol. *Cancer* 1996;**77:**956-963.
17. Jaroszeski MJ, Coppola D, Nesmith G, Pottinger C, Ityacinte M, Benson K, et al. Effects of electrochemotherapy with bleomycin on normal liver tissue in a rat model. *Eur J Cancer* 2001;**37:**414-421.
18. Horikoshi T, Naganuma IT, Ohashi Y, Ueno T, Nakai H. Enhancing effects of electric stimulation on cytotoxicity of anticancer agent against rat and human glioma cells. *Brain Res Bolletin* 2000;**51:**371-378.
19. Kano E, Furakawa FM, Nitta K, Kajimoto KT, Picha P, Sugimoto K, et al. Sensitivities of bleomycin resistant variant cells enhanced by 40ºC hyperthermia in-vitro. *Int J Hyperthermia*1988;**4:**547-553.
20. Shinora H, Hayashi S, Matsumoto H, Kitai R, Ohtsubo T, Nishida T, et al. The effects of combined treatments with low hyperthermia and bleomycin on survival of morine L cells. *J Exp Clin Cancer Res* 1997;**16:**147-152.

21. Hou DY, Hoch S, Johnson GS, Tsou KC, Jones AE, Miller E, et al. A new tumor imaging agent ^{111}In-Bleomycin Complex: Comparison with ^{67}Ga-citrate and ^{57}Co-Bleomycin in tumor bearing animals. *J Surg Oncol* 1984;**27:**189-195.
22. Hou DY, Maruyama Y. Distribution of ^{111}In-Bleomycin complex in small cell lung cancer cells by autoradiography. *J Surg Oncol* 1992;**49:**93-97.
23. Engstrom PE, Person BRR, Salford LG. Dynamic gamma camera studies of ^{111}In-Bleomycin complex in normal and glioma (32, RG2) bearing rats after in vivo electropermeabilization using exponential high voltage pulses. *Bioelectrochem Bioenerg* 1998;**46:**241-248.
24. Grove RB, Eckelman WC, Reba RC. Distribution of labeled bleomycin in normal and tumor-bearing mice. *J Nucl Med* 1973;**14:**917-919.
25. Krohn KA, Meyers JM, Denardo GL, Denardo SG. Comparison of radiolabeled bleomycins and gallium citrate in tumor bearing mice. *J Nucl Med* 1973;**18:**276-281.
26. Robbins PY, Silverstein EB, Fortman DL. The preparation and distribution of ^{111}In-Bleomycin in mice as compared to ^{111}In-Cl_3 for tumor scanning. *J Nucl Med* 1973;**14:**637-639.
27. Brooks RC, Canochan P, Vollano JF, Powel NA, Zwiet J. Metal complexes of bleomycin: Evaluation of [Rh-105]-Bleomycin for use in targeted radiotherapy. *Nucl Med Biol* 1999;**26:**421-430.
28. Thakur ML. The preparation of Indium-111 Labeled bleomycin for tumor localization. *Int J App Rad Iso* 1973;**24:**357-358.
29. Hou DY, Hoch H, Johnson GS, Tsou KC, Jones AE, Farkas RJ, et al. A new ^{111}In-Bleomycin complex for tumor imaging: Preparation, stability and distribution in glioma-bearing mice. *J Surg Oncol* 1984;**25:**168-175.

Address correspondence to: Faraj Tabeie, Department of Medical Physics & Engineering, Shaheed Beheshti University of Medical Sciences. E-mail: tabeie_far@sbmu.ac.ir