Tissue uptakes of ^{67}Ga-bleomycin and carrier free ^{67}Ga in fibrosarcoma-bearing mice

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ABSTRACT

^{67}Gallium-bleomycin complex (^{67}Ga-BLM) was prepared using Thakour method. Radio-thin-layer-chromatography of prepared complex showed A_2 and B_2 radiopeaks with R_f at 0.7 and 0.4 respectively with a purity of above % 95. Tissue uptake of ^{67}Ga-BLM and $^{67}GaCl_3$ in twelve tissues including tumor, blood, liver, lung, spleen, muscle, skin, heart, kidney, colon, colon content ,bladder and the total body were counted by well counter at 1, 2, 4, 24 and 48 hours post injection of radiopharmaceuticals. Uptakes of tissues are expressed as percent injected dose per gram of tissue. The clearance rate of ^{67}Ga-BLM was 1.75-1.95 times faster than $^{67}GaCl_3$ at all time intervals. Bladder uptakes of ^{67}Ga-BLM were highest among twelve tissues at 1,2 and 4 hours after injection, then falling rapidly after 24 and 48 hours. Blood uptake of ^{67}Ga-BLM was lower than $^{67}GaCl_3$ in all time intervals. Colon content uptake of ^{67}Ga-BLM was highest among twelve tissues at 2 and 4 hours post injection. Tumor to tissue activity ratios were also calculated , showing an increase of tumor to blood and muscle ratios. Tumor to blood ratio increased from 0.3 at 1 hour to 5.3 at 48 hours. Activity ratio of muscle increased from 0.5 at 1 hour to 5.5 at 48 hours. Whole body counting of animals showed that effective half lives of ^{67}Ga-BLM and $^{67}GaCl_3$ were about 1 and 15 hours respectively, which renders faster excretion of ^{67}Ga-BLM complex. Biodistribution data clearly indicates that prepared complex in comparison with carrier free ^{67}Ga ($^{67}GaCl_3$) has two main advantages: 1) high tumor to soft tissue uptake ratio that make it suitable for tumor imaging, 2) faster excretion specially at first three hours post injection. In addition complex is stable in vitro and in vivo.

Key words: ^{67}Gallium chloride, ^{67}Gallium-bleomycin, biodistribution, tumor imaging.

Introduction

There has been a growing interest to find new agents for tumor imaging in diagnostic nuclear medicine. So far a few radionuclides i.e. ^{67}Ga, ^{111}In, ^{131}I and ^{169}Yb have been in clinical use with limited application, specially in the case of gasterointestinal cancer and carcinoma of head and neck (1). Since 1962 bleomycin has been

used for chemotherapy because of it's significant antitumor activity against several human malignancies i.e. squamous cell carcinoma, malignant lymphomas, germ cell tumors of testes (2,3), caposi's sarcoma, malignant melanoma (4,5) and head and neck cancers (6). During the past three decades various physical conditions have been used to gain diagnostic and therapeutic applications of bleomycin i.e. hyperthermia (7,8), use of intense electric pulses as electro-chemotherapy (9-11) and labeling of bleomycin with radionuclides (12-22). Several radionuclides such as ^{111}In, ^{131}I, ^{99m}Tc and ^{57}Co have been used to radiolabel bleomycin. The biodistribution of bleomycin complexes in different tumoral models have also been studied (12-22). ^{57}Co-BLM complex was recommended for tumor imaging because it did not bind to transferrin and was stable in-vivo (23-24), but because of it's long physical half life (270 days) it is not favoured for tumor imaging. Preparation , stability and biodistribution of ^{111}In-BLM has also been studied in different tumoral models i.e. glioma, KHJJ tumor, breast mammary adenocarcinoma ,lung cancer and hepatoma in mice and rat (12-21). However different clinical studies have shown that ^{111}In-BLM is not superior to ^{67}Ga-citrate regarding tumor to soft tissue uptake (19). The present study was directed toward assessing a comparative measure of biodistribution of ^{67}Ga-BLM complex and carrier free ^{67}Ga in fibrosarcoma bearing mice. In the earlier report, the preparation, stability and biodistribution of the so called radiopharmaceuticals in normal mice was reported (25).

Materials and Methods

Labelling

Radiopharmaceutical preparation was carried out by labelling the chemotheraputic drug, bleomycin with ^{67}Ga, a radioisotope commonly used in diagnostic nuclear medicine procedures for tumor localization and imaging. ^{67}Ga has physical half life of 78 hours and emits low energy Auger electrons and photons of 93,184 and 296 KeV.

The ^{67}Ga-BLM complex was prepared in optimized conditions as described previously (25). The cyclotron produced $^{67}GaCl_3$ was used for labeling. A total of 0.2-2 ml of ^{67}Ga-Cl_3 at activity of 0.25-2.5 mCi adjusted to pH 2 byl M HCl, was evaporated by slight heating and N_2 gas flow. The ^{67}Ga residue was mixed with 0.25 - 2.5 mg of bleomycin in 0.1 ml of normal saline. The mixture reaction was performed under tight caping of vial and 100 °C for 30 minutes.

Quality control of prepared ^{67}Ga-BLM complex was performed by thin layer chromatography (TLC) on silica gel papers (25,26). One droplet of the complex was placed on a TLC paper (F 1500 / LS, Schleicher & Schoell ®) and the paper was developed in a solution of equal volumes of %10 ammonium acetate and methanol for about 30 minutes. Location of the spots of ^{67}Ga-BLM isomers (A_2 and B_2) at developed silica gel paper were determined by 250 nm UV light for calculation of R_f values. The labeling efficiency was determined by counting 12 cm length of the paper in 10 mm steps by high pure germanium detector system (Cambera ™, GC1020- 7500 SL) .The final solution was then passed through 0.22 μ filter and pyrogen test was performed by a commercial LAL kit.

Stability

A sample of 0.5 mCi prepared ^{67}Ga-BLM complex was kept at room temperature for 24 hours while cheked by radio – thin – layer - chromatography (RTLC) at various time intervals (2, 4, 8, 12 and 24 hours). A 50 μL

sample was taken from shaking mixture and the ratio of free ^{67}Ga to ^{67}Ga-BLM complex was checked by RTLC.

A mixture of 5 parts human serum and one part produced ^{67}Ga-BLM complex (0.2 mCi) was shaked in a 37 °C incubator under nitrogen atmosphere. A 50 µL sample was taken from the shaking mixture every 30 minutes and the ratio of the free ^{67}Ga to ^{67}Ga-BLM was also determined by RTLC method.

Tissue Distribution

Tumor induction was carried out by transplanting 2×10^5 WEHI-164 Balb C fibrosarcoma (prepared from cell bank of Pastour Institute of Iran coded NCBI C200) in the subcutaneous tissue of the left lower quadrant of the abdomen of female Balb C mice weighing 15-20 gr. Animals were kept in isolated animal house and after about four weeks, the diameter of superficial solid tumors reached to about 1 cm.

Total of 40 tumor induced mice were selected for tissue distribution studies. Twenty mice in five equal population groups received approximately 40 µCi of ^{67}Ga-BLM complex ($\leq$ 3 µg bleomycin) via the dorsal tail vein. The mice were sacrificed at 1,2,4,24 and 48 hours post injection and samples of 12 selected tissues including tumor, blood, liver, lung, spleen, muscle, skin, heart, kidney, colon, colon content and bladder were excised, weighted wet and counted in a NaI (Tl) well counter (Capintec, CRC-15R). The average of percent injected dose per gram of tissue for 4 mice and the standard deviation have been reported. Other 20 tumoral mice received 40 µCi of ^{67}Ga-Cl_3 and the above procedure was repeated. Tissue distribution of ^{67}Ga-BLM and ^{67}Ga-Cl_3 radiopharmaceuticals were repeated for 40 normal Balb C mice in comparison with tumoral animals.

One group of four tumoral mice received 40 µCi of ^{67}Ga-BLM and another identical group received same amount of ^{67}Ga-Cl_3. Animals then underwent whole body activity counting in cone shaped plexyglass restrainer using a well counter at 1, 2, 4, 24 , 48 and 72 hours post injection.

Results

Labelling and Stability

Radio thin layer chromatography of ^{67}Ga-BLM showed two distinct radio peaks of A_2 and B_2 with R_f of 0.7 and 0.4 respectively. Radiochomatogram scans of TLC plates showed radiochemical purity of higher than 95% (Fig 1).

The stability of complex in-vitro was checked by RTLC method. The bounded to free gallium ratio remained constant up to 24 hours after labeling. The stability of complex unchaged also in presence of serum proteins of mice and human didn't change. Radiochemical purity also remained constant (95%) even at tempretures up to 95°C. The pyrogen test with LAL showed no detectable contamination.

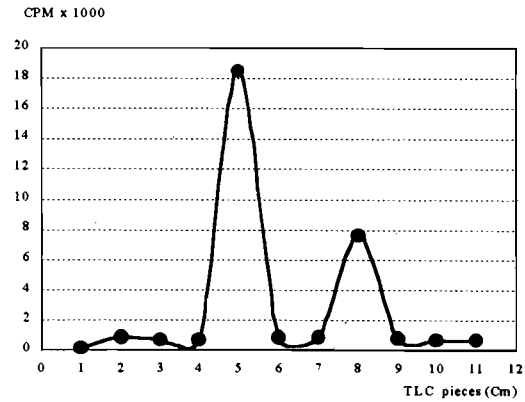

Fig. 1. Radiochromatoscan of ^{67}Ga-BLM complex

Tissue Distribution

The distribution and retention of ^{67}Ga-BLM and ^{67}Ga-Cl_3 among 12 tissues of fibrosarcoma-bearing mice 1, 2, 4, 24 and 48 hours after injection of radiopharmaceuticals are shown in table 1. The uptake amount and tissue

concentration of pharmaceuticals are given as mean percent injected dose per gram of tissue. In addition, the standard deviations (n=4) are also given as an indication of the spread at data. The concentrations of ^{67}Ga-BLM and $^{67}GaCl_3$ in 6 tissues at five selected time intervals after injection are compared in figures 2 and 3.

Tissue activity counting by well counter (table 1 and figures 2 and 3) showed that bladder uptake (% dose /gr tissue) of ^{67}Ga-BLM at 1, 2 and 4 hours after injection were about 3-10 times higher than other 11 tissues. Bladder uptake of $^{67}GaCl_3$ were about 1.82-3.62 times lower than ^{67}Ga-BLM at first three times after injection and decreased by time. Blood uptakes showed monophasic decreasing patterns for both radioparmaceuticals, but the blood uptakes of $^{67}GaCl_3$ are about 1.07-2.64 times higher than ^{67}Ga-BLM among five time intervals after injection.

Liver and lung uptakes from two radiopharmaceuticals showed biphasic patterns with two distinct increasing and decreasing phases. Liver uptake of ^{67}Ga-BLM increases from 2.97 ± 0.11 at 1hour after injection and reaches to a maximum of 3.56 ±0.45 after 4 hours and then falls to 2.61±0.97 after 48 hours. Liver uptake of $^{67}GaCl_3$ also had two increasing and decreasing phases. Liver uptake of $^{67}GaCl_3$ also had two increasing and decreasing phases, but it`s maximum was at 24 hours after after injection and at this time the uptake of $^{67}GaCl_3$ was significantlly higher than^{67}Ga-BLM. Lung uptake of two radiopharmaceuticals reached to maximum after 2 hours post injection

Muscle uptake of two radiopharmaceuticals showed monophasic decreasing patterns, but clearance rate of ^{67}Ga-BLM was about two times faster than $^{67}GaCl_3$ Uptake and concentration of colon content had second rank among tissues at first three times after injection, which shows gastrointestinal secretion of ^{67}Ga-BLM.

Table 1. Distribution of ^{67}Ga-BLM and $^{67}GaCl_3$ in tumoral mice

Time (hrs)		1		2		4		24		48	
Organ		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tumor	Ga-BLM	1.21	0.54	2.86	0.93	3.56	0.98	3.79	1.92	4.63	1.71
	Ga-Cl3	1.12	0.69	2.32	0.78	4.16	1.31	7.21	1.86	5.91	1.36
Blood	Ga-BLM	3.91	0.71	3.85	0.83	2.43	0.41	0.97	0.21	0.87	0.13
	Ga-Cl3	6.12	1.19	4.14	0.83	3.04	0.92	2.61	0.52	1.88	0.12
Liver	Ga-BLM	2.98	0.11	3.11	0.54	3.56	0.45	2.81	0.67	2.61	0.97
	Ga-Cl3	2.87	0.52	3.18	0.76	3.51	0.56	4.63	0.66	3.21	0.72
Lung	Ga-BLM	3.2	0.91	3.54	0.82	3.02	0.43	2.51	0.34	2.14	0.55
	Ga-Cl3	4.41	1.95	5.31	1.06	3.85	0.98	3.41	0.14	2.25	0.16
Spleen	Ga-BLM	2.1	0.12	2.17	0.19	2.32	0.23	0.95	0.36	0.86	0.78
	Ga-Cl3	2.75	0.21	3.11	0.39	3.86	0.57	4.52	0.77	4.87	0.89
Muscle	Ga-BLM	2.36	0.11	1.72	0.1	1.32	0.16	0.84	0.14	0.83	0.13
	Ga-Cl3	2.41	0.72	2.45	0.89	2.41	0.46	2.23	0.14	1.63	0.13
Skin	Ga-BLM	1.76	0.09	2.56	0.15	2.81	0.21	2.92	0.17	2.36	0.11
	Ga-Cl3	2.86	0.67	3.12	1.06	3.63	1.13	2.81	0.84	2.37	0.76
Heart	Ga-BLM	1.12	0.12	1.11	0.14	1.05	0.21	0.84	0.18	0.71	0.12
	Ga-Cl3	2.31	0.13	2.16	0.15	2.32	0.22	2.13	0.19	1.46	0.19
Kidney	Ga-BLM	3.82	0.87	2.65	0.89	2.16	0.31	1.89	0.21	1.64	0.17
	Ga-Cl3	3.72	0.88	2.78	0.34	2.91	0.23	2.74	0.14	2.45	0.18
Colon	Ga-BLM	3.11	0.61	0.63	0.41	2.67	0.51	1.11	0.19	0.92	0.22
	Ga-Cl3	2.32	0.41	2.31	0.24	2.46	0.31	1.82	0.11	1.63	0.08
Colon content	Ga-BLM	4.63	1.12	5.72	1.31	6.11	1.25	1.52	0.12	1.37	0.08
	Ga-Cl3	3.61	0.17	4.51	0.15	3.89	0.31	1.98	0.08	1.82	0.13
Bladder	Ga-BLM	11.23	2.34	10.56	2.15	9.7	1.72	3.22	0.74	2.75	0.19
	Ga-Cl3	5.93	1.51	2.91	0.81	2.13	0.85	1.85	0.51	1.41	0.41

Activity ratios of tumor to five tissues including blood, liver, lung, muscle and bladder are shown in figures 4 and 5 and for all tissues at two times (24 and 48 hours) after injection are shown in figures 6 and 7 for both radiopharmaceuticals.

Activity ratios of tumor to five tissues for both radio pharmaceuticals increased

with exception of liver at only one hour after. Tumor to blood and muscle ratios of ^{67}Ga-BLM at all time intervals were higher than $^{67}GaCl_3$. Lung uptake of two radiopharmaceuticals reached to maximum after 2 hours post injection.

Whole body counting of animals by well counter at 1, 2, 4, 24, 48 and 72 hours post injection are shown as percent of total activity administered(40 μCi) for both radiopharmaceuticals in figure 8.

The clearance rate and washout of ^{67}Ga-BLM is about 2 times faster than $^{67}GaCl_3$. The effective half lives of ^{67}Ga-BLM and $^{67}GaCl_3$ were calculated to be about 1 and 15.5 hours in tumoral model respectively. Regarding the physical half life of ^{67}Ga (78 hours), the biological half lives of ^{67}Ga-BLM and $^{67}GaCl_3$ were calculated to be about 1 and 18.9 hours respectively.

Discussion

In search to find a new valuable radiopharmaceutical for tumor imaging and therapy, this paper reports the optimum labeling of bleomycin with ^{67}Ga and the biodistribution of the produced complex (^{67}Ga-BLM) in fibrosarcoma-bearing mice.^{67}Ga in the form of ^{67}Ga-citrate has been used in nuclear medicine for tumor imaging for some time.

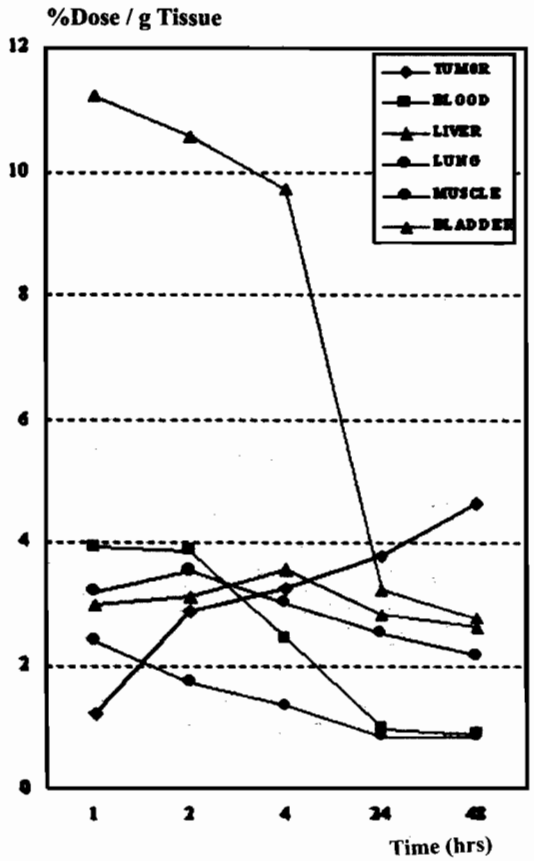

Fig. 2. Tissue distribution of ^{67}Ga-BLM in tumoral mice

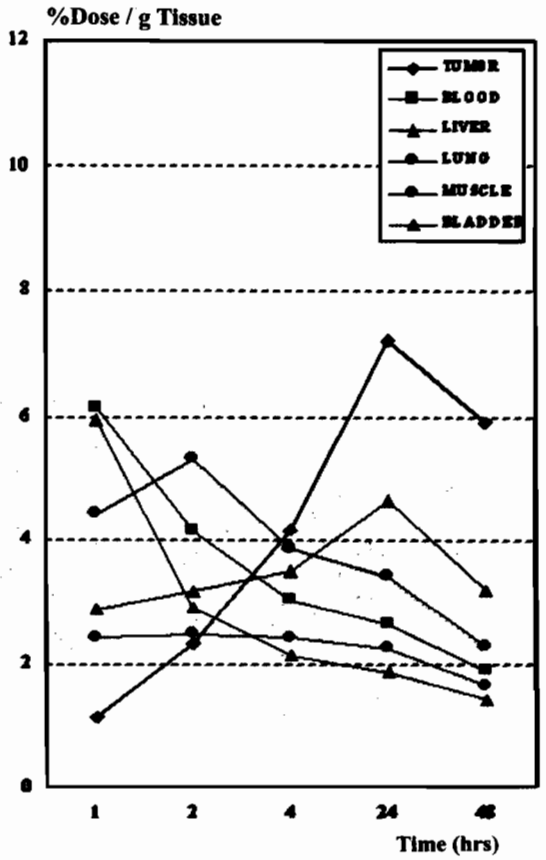

Fig. 3. Tissue distribution of $^{67}GaCL_3$ in tumoral mice

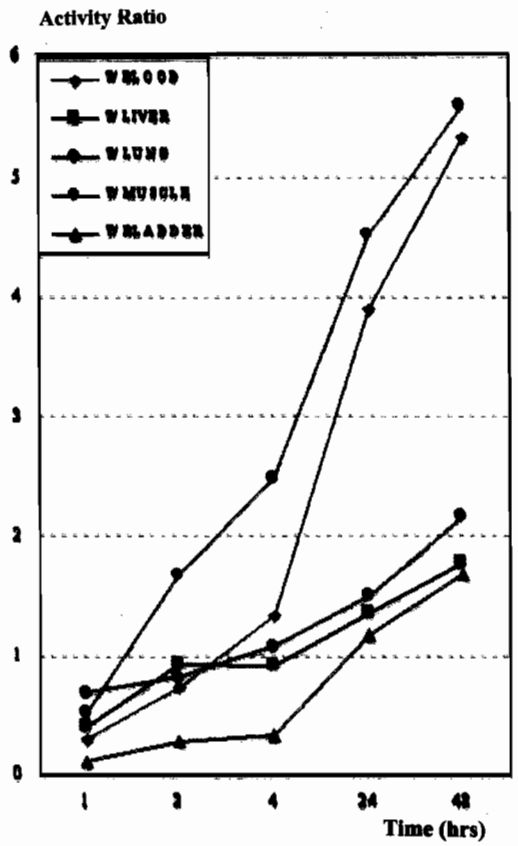

Fig. 4. Activity ratios of tumor to tissue for ^{67}Ga-BLM

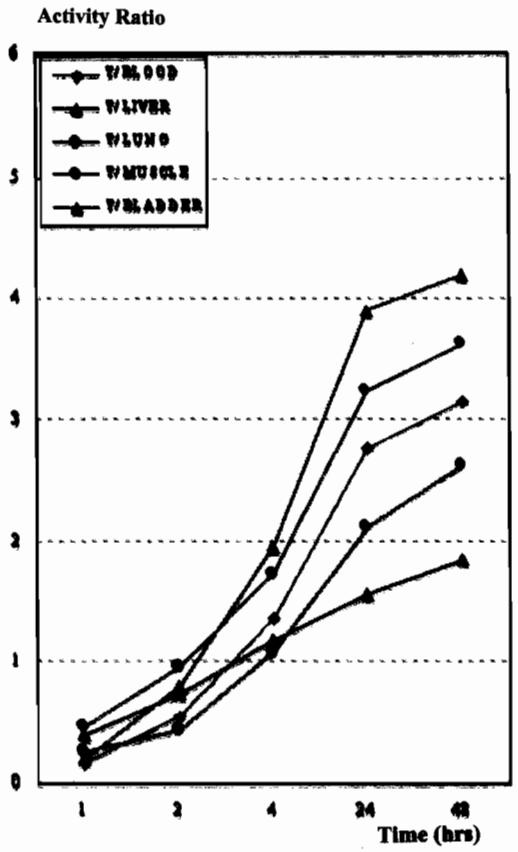

Fig. 5. Activity ratios of tumor to tissue for $^{67}GaCl_3$

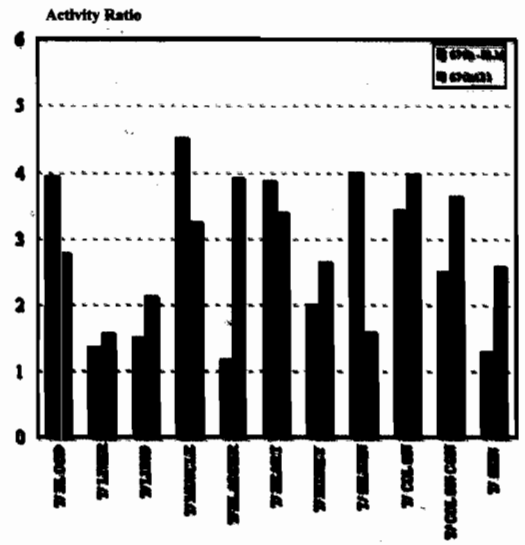

Fig. 6. Activity ratios of tumor to tissue at 24 hours

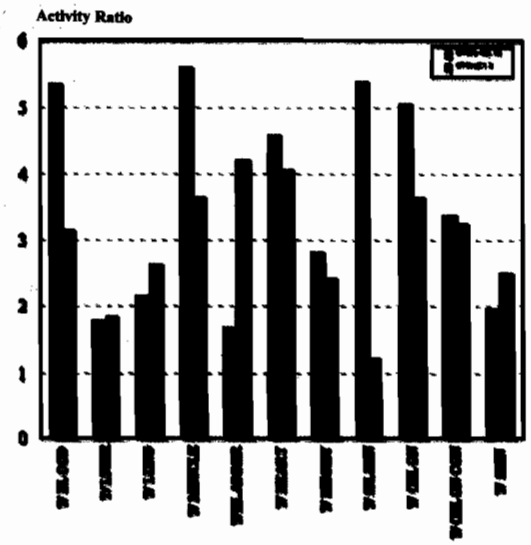

Fig. 7. Activity ratios of tumor to tissue at 48 hours

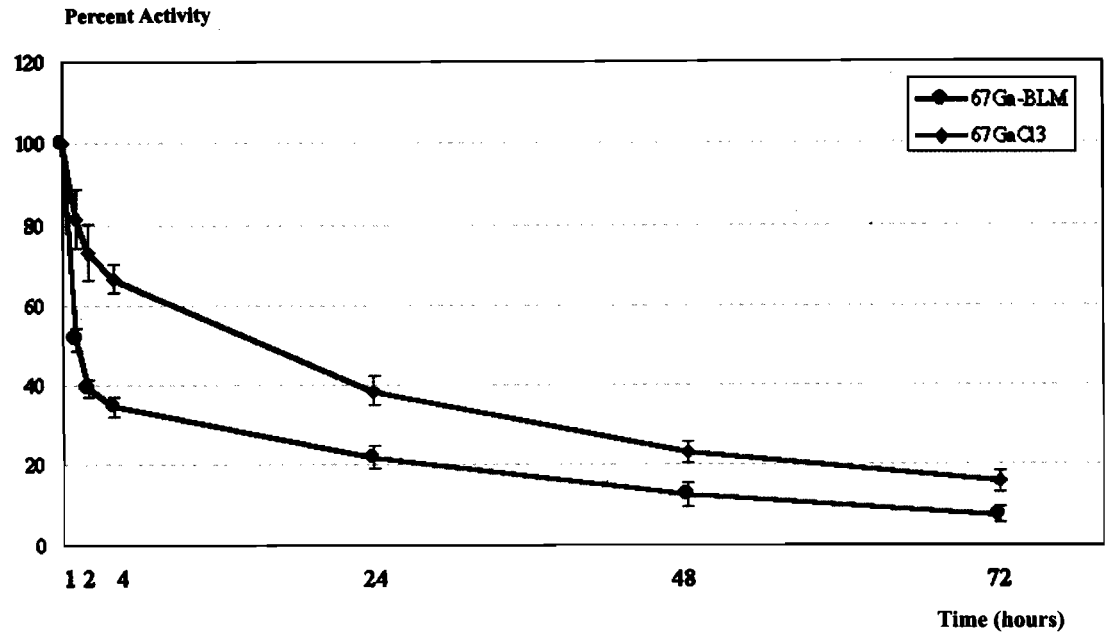

Fig. 8. Percent of whole body retained activity after iv injection of radiopharmaceuticals

However, it had three main disadvantages: 1) high excretion in GItract causing a difficault clinical interpretation, 2) slow clearance rate from blood and GI tract in dictating a long time for imaging after administration, 3) low degree of sensitivity and specifity for detection of certain tumor types i.e. adenocarcinoma. The binding of chemotheraputic drug bleomycin having high tumor affinity, with tumor localizing agents such as ^{111}In and ^{67}Ga could be altered in tissue distribution when administered in tumoral mice.

Thin layer chromatography of produced ^{67}Ga-BLM complex showed only two major spots (Rf =0.4 and 0.6) containing 95% of total activity. When preparing ^{67}Ga-BLM, the pH must be kept around 2. This value certainly differs from those of 4.0-4.5 and 6.5 which were used for preparing of ^{111}In-BLM (8,9) and ^{67}Ga-BLM complex respectively (8-10). However the pH value of 2 is in full agreement with other investigators that used low pH between 2-3 for preparation of ^{111}In-BLM and ^{111}In-BLMC (11-14).

As mentioned earlier, the biodistribution study of two radiopharmaceuticals (^{67}Ga-BLM and $^{67}GaCl_3$) were carried out by sacrifing of animals and counting activity of wet tissues as percent of administered activity per gram of tissue.

Blood uptake of ^{67}Ga-BLM at all time intervals were lower than $^{67}GaCl_3$ but, activity ratios of tumor to blood and muscle of ^{67}Ga-BLM was significantly higher than $^{67}GaCl_3$ and gradually increased with time, which is in agreement with ^{111}In-BLM and ^{111}In-BLMC complexes (2-8). Higher blood uptake of $^{67}GaCl_3$ is most likely due to transferrin binding of carrier free $^{67}Ga^{+3}$, hence lower blood uptake of ^{67}Ga-BLM complex can be considered as non-transferrin binding and stability *in-vivo*.

Higher bladder uptake and faster excretion of ^{67}Ga-BLM complex in comparison with $^{67}GaCl_3$ is in agreement with studies comparing washout of ^{111}In-BLM complex and carrier free $^{111}In^{+3}$ and $^{67}Ga^{+3}$ (12,13).

There are considerable similarities between colon content uptake patterns of both radioparmaceuticals in tumoral and normal mice models showing high uptakes specially at first three hours after injection (18,25), which is in agreement with high GI tract secretion of ^{67}Ga in clinical clinical practice(19).

Biodistribution data clearly indicate that prepared complex (^{67}Ga-BLM) in comparison with carrier free ^{67}Ga ($^{67}GaCl_3$) had two main advantages: 1) high tumor to soft tissue uptake ratio that makes it suitable for tumor imaging , 2) faster excretion at first three hours post injection. These findings suggest that the produced complex could be a good choice for tumor detection in diagnostic nuclear medicine .

Tumor detection properties of ^{111}In-BLM and ^{57}Co-BLM were compared with ^{67}Ga-citrate in patients with various malignancies and it was concluded that ^{111}In-BLM is not superior to ^{67}Ga-citrate for tumor detection (20), hence further studies is suggested for clinical evaluation of ^{67}Ga-BLM in comparison with routinly used ^{67}Ga-citrate for tumor detection and imaging purposes (further study is in progress).

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