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# Production and Evaluation of Lutetium-177 Maltolate as a Possible Therapeutic Agent

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**Abstract:** Development of oral therapeutic radiopharmaceuticals is a new concept in radiopharmacy. Due to the interesting therapeutic properties of $^{177}$Lu and oral bioavailability of maltolate (MAL) metal complexes, $^{177}$Lu-maltolate ($^{177}$Lu-MAL) was developed as a possible therapeutic compound for ultimate oral administration. The specific activity of 2.6-3 GBq/mg was obtained by irradiation of natural $Lu_2O_3$ sample with thermal neutron flux of $4\times10^{13}$ n.cm$^{-2}$.s$^{-1}$ for Lu-177. The product was converted into chloride form which was further used for labeling maltol (MAL). At optimized conditions a radiochemical purity of about >99% was obtained for $^{177}$Lu-MAL shown by ITLC (specific activity, 970-1000 Mbq/mmole). The stability of the labeled compound as well as the partition coefficient was determined in the final solution up to 24h. Biodistribution studies of Lu-177 chloride and $^{177}$Lu-MAL were carried out in wild-type rats for post-oral distribution phase data. Lu-MAL is a possible therapeutic agent in human malignancies for the bone palliation therapy so the efficacy of the compound should be tested in various animal models.

***Keywords:*** ***Maltolate, Lu-177, Biodistribution, Radiolabeling***

## 1- Introduction

Many $\beta^-$-emitters such as Sm-153, Ho-166 and Lu-177 can be produced in reasonable amounts using (n, gamma) reactions. Due to the half-life limitations in the application of mentioned radionuclides the emerging need for a long half-life beta emitter such as lutetium-177 is obvious.

Owing to lutetium-177 suitable decay characteristics [$T_{1/2}$ = 6.73 d, $E\beta_{max}$ = 497 keV, $E\gamma$ = 112 keV (6.4%), 208 keV (11%)] [1] as well as the feasibility of large-scale production in adequate specific activity and radionuclidic purity using a moderate flux reactor, $^{177}$Lu has been considered as a promising radionuclide for developing therapeutic radiopharmaceuticals [2].

Maltol (3-Hydroxy-2-methyl-4-pyron) is commonly formed when sugars are heated. Maltol loses its hydroxyl proton at neutral to basic pH levels, forming the maltolate anion; this anionic molecule forms a strong bidentate/tridentate chelate with gallium, iron, zinc, aluminum, vanadium and many other metals [3] (Fig. 1.).

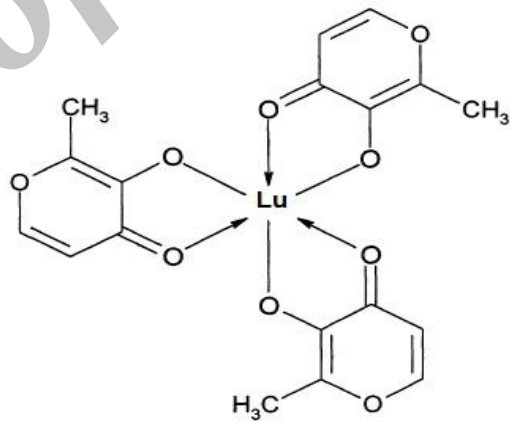


**Fig. 1.** Structural formula of lutetium maltolate.

Most of maltolate metal complexes are reported as biologically active compounds. Gallium-maltolate, is a superior antitumor oral drug in clinical trial phases in contrast to gallium nitrate as well as many other metal based antitumural compounds. Ga-maltolate is an effective anti-lymphoma compound with activity against Ga nitrate-resistant lymphomas [4] and urothelial malignancies [5]. A more recent clinical trial confirmed the efficacy of gallium nitrate in patients with non-Hodgkin's lymphoma whose disease had relapsed

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following treatment with conventional chemotherapeutic agents [6]. The chemistry and pharmacokinetics of Ga-maltolate have been extensively reported before starting the clinical phase [7] and also the pharmacokinetic has been shown in animals after oral administration [8]. On the other hand, aluminum-maltolate complex has also demonstrated in vitro apoptotic cell death pathway in man [9] as well as anti-microbial effects [10]. Mixed copper-maltolate complexes have also been reported for high cytotoxicity against HeLa (cervical) cancer cell lines demonstrating a synergistic effect between the metal and ligand in the cell death [11].

Intravenous injection is a major route of administration of radiopharmaceuticals which would impose various safety and quality assurance aspects leading to increased expenses and limitations. Although I-131 oral capsules and solutions have been used as therapeutic agents for some time, no other examples of oral therapeutic radiopharmaceuticals exist.

Since maltol-metal complexes are non-toxic, highly lipophilic, non-allergic and safe compounds having high oral bioavailability, the development of possible oral radiopharmaceuticals seem possible using these agents. Due to the success of oral gallium maltolate preparations and the importance of the bone pain palliation therapy in various metastatic carcinomas around the world, we focused on the development of a possible oral radiopharmaceutical for the bone pain palliation therapy. In this work, we report preparation as well as stability tests and biodistribution of $^{177}$Lu-MAL as a potential therapeutic complex for determination of the bone uptake.

Although the compound should be finally administered orally, the phase II of distribution is a parantheral part, thus this study has included the vascular distribution part for better judgement.

## 2. Experimental Methods

Maltol was purchased from Aldrich Co., Germany, without further purification. Chromatography paper (Whatman No. 2) was obtained from Whatman (Maidstone, UK). Radio-chromatography was performed using a bioscan AR-2000 radio TLC scanner (Bioscan, Paris, France). A high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) were used for counting distributed activities in rat organs. All other chemical reagents were purchased from Merck (Darmstadt, Germany). Calculations were based on the 112 keV peak for $^{177}$Lu. All values were expressed as mean ± standard deviation (Mean± SD) and the data were compared using Student's T-Test. Statistical significance was defined as $P<0.05$. Animal studies were carried out in accordance with The United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd ed. Male healthy rats were purchased from Pasteur Institute, Tehran, Iran.

## 3. Production and Quality Control of $^{177}LuCl_3$ Solution

$^{177}$Lu was produced by irradiation of natural $Lu_2O_3$ target (1 mg) at a thermal neutron flux of approximately $4\times10^{13}$n/cm$^2$.s for 5 days at Tehran Research Reactor (TRR) according to the reported procedures [12]. The irradiated target was dissolved in 200µl of 1.0 M HCl, to prepare $^{177}LuCl_3$ and diluted to the appropriate volume with ultra pure water, to produce a stock solution of final volume of 5ml. The mixture was filtered through a 0.22µm biological filter and sent for use in the radiolableing step. For radionuclidic purity determination, the samples were checked by gamma-ray spectroscopy on an HPGe detector for 5 min basing on two major photons of $^{177}$Lu (6.4% of 0.112 MeV and 11% of 0.208 MeV). The radiochemical purity of the $^{177}LuCl_3$ was checked using 2 solvent systems for ITLC [A: 10mM DTPA pH.4 and B: ammonium acetate 10%: methanol (1:1)] for parallel determination of colloids as well as other ionic species.

## 4. Labeling of Maltolate with $^{177}LuCl_3$

Most of the papers have reported the maltolate metal complex synthesis in aqueous phase [3]. However, we made the synthesis in ethanolic media. Briefly, $^{177}LuCl_3$ (3mCi, 0.1 ml) was

added to a borosilicate vial and dried by heating (50°C) under a nitrogen flow for about 15 minutes. Then, maltol (31mg, 0.25 mmol) dissolved in absolute ethanol (1 ml) was added to the dried residue and the mixture was agitated and incubated at 60°C for 2 hours. The radiochemical purity of free Lutetium and Lu-MAL was determined by counting Whatman No. 2 sheets as stationary phase using various mobile phases (A: ammonia: water: methanol (2: 40: 20), B: 1mM DTPA aqueous solution, C: %10 ammonium acetate: methanol system, 1:1). After obtaining the desired radiochemical purity, the ethanolic solution was concentrated by warming 40-50°C to 0.05ml and then diluted to a 5% solution by adding 1ml of normal saline.

## 5. Stability Testing of the Radiolabeled Compound in Aqueous Solution

Stability of $^{177}$Lu-MAL in final preparation was determined by storing the final solution at 25ºC for 24 h and performing frequent ITLC analysis using ammonia: water: methanol (2:40:20) mobile phase to determine radiochemical purity.

## 6. Stability of $^{177}$Lu-MAL in Presence of Human Serum

Final $^{177}$Lu-MAL solution (200µCi, 50 µl) was incubated in the presence of freshly prepared human serum (300 µl) and kept at 37ºC for 2 days. The complex stability was assessed by performing frequent ITLC analysis using ammonia: water: methanol (2:40:20) mobile phase to determine radiochemical purity.

## 7. Determination of Partition Coefficient

The partition coefficient of the $^{177}$Lu-MAL was measured following 1 min of vigorous vortex mixing of 1 ml of 1-octanol and 1 ml of isotonic actetate-buffered saline (pH=7) with approximately 3.7 MBq (100µCi) of the radiolabeled complex at 37°C. Following further incubation for 5 min, the octanol and aqueous phases were sampled and counted in an automatic well counter. A 500µl sample of the octanol phase from this partitioning was repartitioned two or three times with fresh buffer to ensure that traces of hydrophilic $^{177}$Lu impurities did not alter the calculated P values. The reported log P values are the averages of the second and third extractions from three to four independent measurements, log P values represent the mean (standard deviation) of five measurements.

## 8. Biodistribution of $^{177}$Lu-Maltolate and $^{177}LuCl_3$ in normal rats

To determine comparative biodistribution, $^{177}$Lu-MAL and $^{177}LuCl_3$ were administered to the normal rats in separate groups (n=3). A volume (100-120µl) of final $^{177}$Lu-MAL solution (130±5µCi) radioactivity was injected intravenously to the rats through their tail vein. The animals were sacrificed at the exact time intervals (2, 4, 24 hours and 7 days) and specific activity of each organ was calculated as percentage of injected dose per gram using HPGe detector.

## 9. Results and Discussion

### *9.1 Production and Quality Control of $^{177}$Lu*

The radionuclide was prepared in a research reactor according to the regular methods within the range of specific activity 2.6-3 GBq/mg for radiolabeling use. The obtained radionuclidic purity was 99.98% (Fig. 2).

The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated for obtaining the desired pH and volume followed by sterile filtering. The radiochemical purity of the $^{177}$Lu solution was checked in two solvent systems on Whatman No. 2 papers in 10mM DTPA, free $Lu^{3+}$ cation was chelated to the more lipophilic LuDTPA form and migrated to higher $R_f$, while small radioactive fraction remained at the origin which could be related to other Lu ionic species, not forming LuDTPA complex, such as $LuCl_4^-$, etc. and/or colloids (4%).

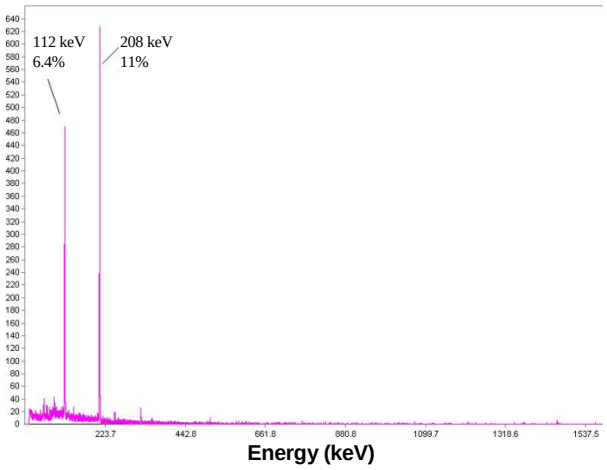


**Fig. 2.** Gamma-ray spectrum for Lu-177 chloride solution used in this study.

On the other hand, 10% ammonium acetate: methanol mixture was also used for the determination of radiochemical purity. In this solvent system, the fast eluting species were possibly Lu-177 cations, other than $Lu^{3+}$ (2%) and the remaining fraction at $R_f$.0 was a possible mixture of $Lu^{3+}$ and/or colloids. The difference in values of impurity in two solvent systems was possibly due to the presence of colloidal impurity in the sample (2%).

## 10. Preparation of $^{177}$Lu-MAL

In order to obtain the highest specific activity in the shortest possible time, a quantitative study was designed using different amounts of MAL and various time intervals for a specific amount of radioactivity (2mCi of $LuCl_3$ for instance) while 50ºC was considered a suitable temperature. A satisfactory labeling yield of higher than 99% was obtained at this temperature using 18-22mg of MAL within 2 h.

Because of relative lipophilic $^{177}$Lu-MAL complex and participation of several polar functional groups in its structure, $^{177}$Lu-MAL migrated to the solvent frontline in ITLC ($R_f$.08) while $^{177}$Lu cation was retained at the origin (Rf. 0.05) in a mixture of ammonia: water: methanol (Fig. 3).

As shown in Fig. 1. $^{177}$Lu-MAL is majorly prepared in a 3:1 ligand: cation ratio 2 components with reported ratio mixture, considering the molar ratio, a molecular weight of 552 can be calculated for $^{177}Lu(MAL)_3$, resulting in a specific activity of 970-1000 Mbq/mmole under optimized radiolabeling conditions. The labeling step took about 2 h. In all radiolabeling procedures (n=5), the labeling yield was over 99%.

The partition coefficient for the labeled compound was calculated (logP. 1.869) demonstrating a rather lipophilic complex as it could be observed from the chromatographic behavior.

The final radiolabeled complex diluted in normal saline was then passed through a 0.22µm (Millipore) filter for sterilization. Incubation of $^{177}$Lu-MAL in freshly prepared human serum for 24 h at 37°C showed no loss of $^{177}$Lu from the complex (less than 0.1%).

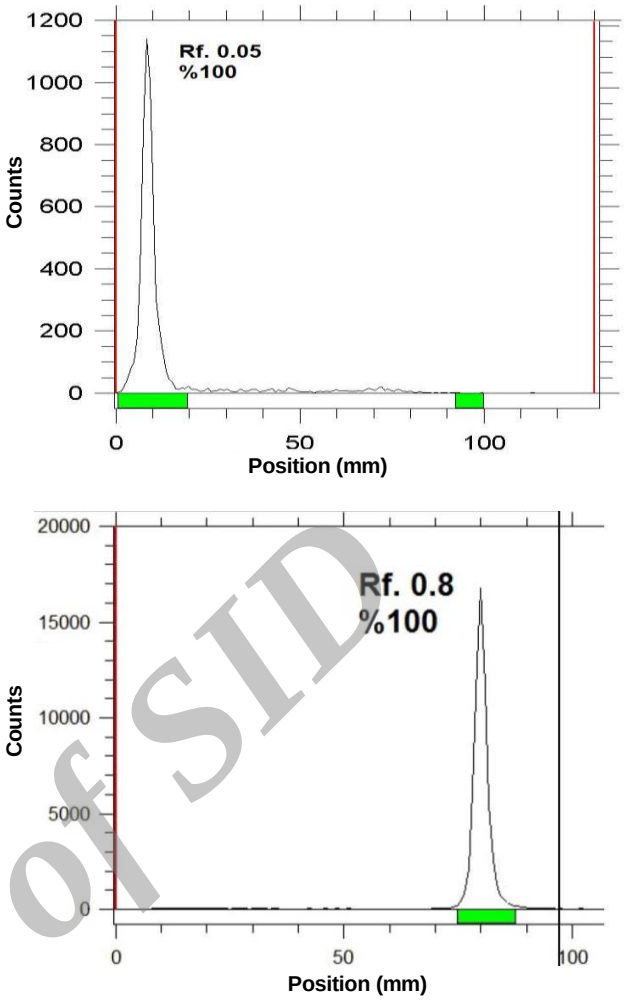


**Fig. 3.** Radio chromatogram of free $Lu^{3+}$ cation (up) and $^{177}$Lu-maltolate (down) using a mixture of ammonia: water: methanol 2h after labeling.

## 11. Biodistribution studies for free $^{177}$Lu cation in rats

The animals were sacrificed by $CO_2$ asphyxiation at selected times after injection. Dissection began by drawing blood from the aorta followed by removing the heart, spleen, muscle, bone, kidneys, liver, intestine, stomach, lungs and skin samples. The tissue uptakes were calculated as the percent of area under the curve of the related photo peak per gram of tissue (% ID/g) (Fig. 4).

The liver uptake of the cation is comparable with many other radio-lanthanides mimicking calcium cation accumulation; about %3 of the activity accumulates in the liver after 48 h. The transferin-metal complex uptake and the final liver delivery seem the possible route of accumulation.

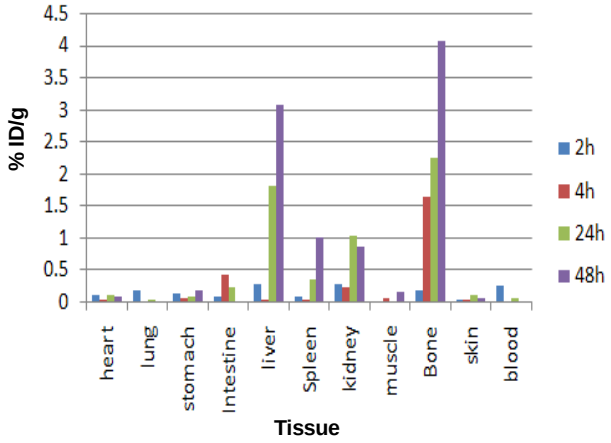


**Fig. 4.** Percentage of injected dose per gram (ID/g %) of $^{177}LuCl_3$ in wild-type rat tissue at 2, 4, 24 and 48h post injection.

The blood content is low at all intervals which shows the rapid removal of activity in the circulation. The lung, muscle and also skin do not demonstrate significant uptake which is in accordance with other cation accumulation. A %4 bone uptake is observed for the cation which remains almost constant after 48 h (data not shown). The spleen also has significant uptake possibly related to reticuloendothelial uptake. The kidney plays an important role in $^{177}$Lu cation excretion especially after 24 h.

The accumulation of $^{177}$Lu-MAL is demonstrated in Fig. 5. The bone, liver and kidney were the major accumulation sites of the radiolabeled compound. A major route of excretion for the tracer was the urinary tract.

Comparison of vital organ uptake for $^{177}LuCl_3$ and $^{177}$Lu-MAL demonstrates kinetic pattern difference for both species. $^{177}$Lu cation is accumulated in the liver in the first 24h post injection, and it can be assumed that later on, the activity is excreted from the liver via the biliary tract, while $^{177}$Lu-MAL is excreted through the kidneys with an exponential rate in 5 days (Fig. 6).

As shown in Fig. 7. 24 h after injection free Lu-177 accumlates in the bone (2.2%), while in case of $^{177}$Lu-MAL, the uptake is significantly higher (3.5%) thus at least 70% more uptake is observed for the labeled compound. On the other hand, according to many published and unpublished data on the most important Lu-177 bone pain palliation complex, $^{177}$Lu-EDTMP accumulates in this tissue up to the maximum of 2.5-3% which shows even less uptake compared with that of $^{177}$Lu-MAL.

In case of the kidney, as shown in Fig. 7, free Lu-177 is exctreted through the kidney in a linear manner and this uptake is rapidly decreased after 24h, while in case of $^{177}$Lu-MAL, the uptake increases after 24h (Fig. 7).

As shown in Fig. 8. 4h after injecting the two spieces the bidistributions show a different pattern while in 2-24 h the labeled compound is not significantly accumulated in the liver (0.6-0.8%), whereas the cation itself is drastically accumulated in the liver up to 1.8%.

However, the oral absorption of both compounds should be tested in appropriate animal models. Also compared to analogous Ga-maltolate, the biological evaluation of $^{177}$Lu-MAL upon tumor models can be carried out.

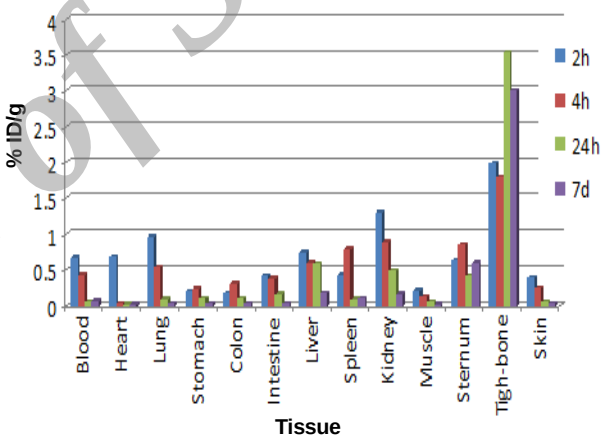


**Fig. 5.** Percentage of injected dose per gram (ID/g %) of $^{177}$Lu-MAL in wild-type rat tissue at 2, 4, 24 and 7d post injection.

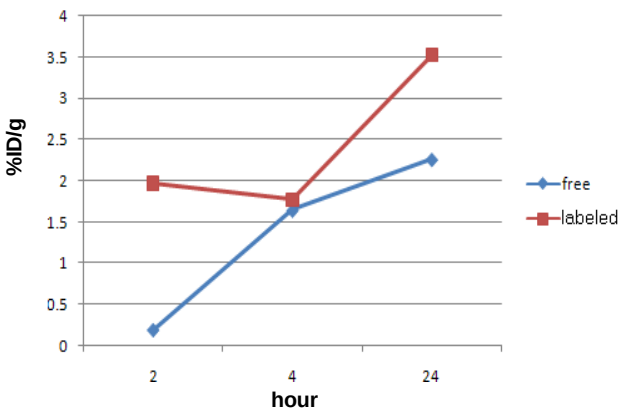


**Fig. 6.** Comparative %ID/g in the bone tissue for $^{177}$Lu-MAL (labeled) and $^{177}LuCl_3$ (free) in wild-type rats.

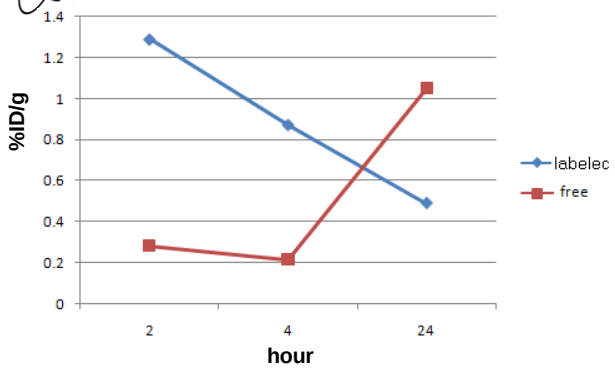


**Fig. 7.** Comparative %ID/g in the kidney tissue for $^{177}$Lu-MAL (labeled) and $^{177}LuCl_3$ (free) in wild-type rats.

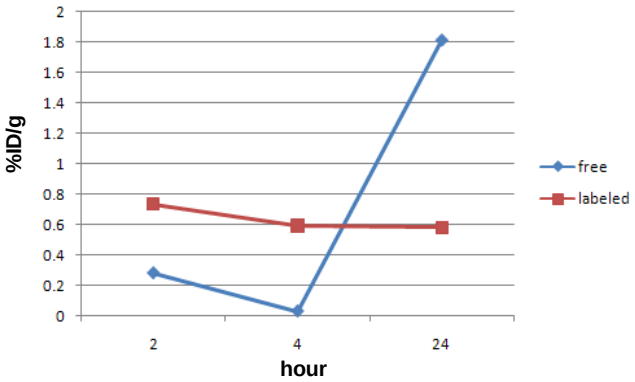


**Fig. 8.** Comparative %ID/g in the liver for $^{177}$Lu-maltolate (red) and $^{177}LuCl_3$ (blue) in wild-type rats.

## 12. CONCLUSION

Under optimized conditions, total labeling and formulation of $^{177}$Lu-MAL took about 2 h, with a radiochemical yield higher than %99. The radio-labeled complex was stable in aqueous solutions for at least 48 hours and no significant amount of other radioactive species was detected by ITLC. Trace amounts of $^{177}LuCl_3$ (≈1%) were detected by ITLC. Specific activity calculated for the radiolabeled compound was 970-1000 Mbq/mmole. The biodistribution of labeled compound was checked in wild-type rats up to 24 h and a significant accumulation took place in the bone with significantly less accumulation in the liver compared with free Lu-177 cation. Although $^{177}$Lu-EDTMP demonstrated less bone uptake (%2-3) than $^{177}$Lu-MAL (%3.5) in 24h, however for $^{177}$Lu-MAL, critical organ uptake (kidneys and lung), it was higher. On the other hand, heavy metal bisphosphonates (such as $^{177}$Lu-EDTMP) were not well absorbed through GI and could not be used as oral drugs, while $^{177}$Lu-MAL could be a good candidate for oral administration. The exact oral administration studies of $^{177}$Lu-MAL must be achieved upon appropriate animal model in the next step.

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