Preparation and Preliminary Biological Evaluation of ^{177}Lu-DOTA folate as Potential Folate Receptor Targeting Therapeutic Agent

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1. Introduction

Folic Acid (FA) and FA derivatives are overexpressed on several tumor cells [1,2].The cell-membrane folic acid receptors are known to be responsible for the cellular accumulation of FA and FA analogs, such as methotrexate and folic acid. Folate has been characterized to have high affinity for the folate-receptor positive cells and tissues and considered to be useful as diagnostic imaging and therapeutic agent. In 1940s, Folate analogue, aminopterin, was first used for treatment of leukemia and recently, many folate derivatives were tried for cancer-treatment agent as well as visualization of folate receptor. Many researchers tried to conjugate folic acid with macromolecules or low molecular weight chelators through its alpha or gamma carboxylate[1,2,3,4]. However, despite the reduced binding affinity, FAs are still recognized by the folate receptor. Therefore, we focused to develop folate-based radiopharmaceutical that has the potential to be used as a therapeutic agent. We report here the synthesis and the radiolabeling of ^{177}Lu-DOTA as well as the biodsitribution data of our developed compound.

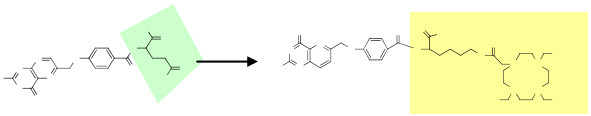

Fig. 1 Folic acid and its derivatives containing DOTA

2. Methods and Results

All chemicals were purchased from Aldrich and used without any further purification. All the processes of the reaction were identified with TLC and NMR. $^{177}LuCl_3$ was produced by the KAERI (Korean Atomic Energy Research Institute). The labeling yield and radiochemical purity were determined by Radio-HPLC using X-Terra C-18 reversed phase column with eluent system consisting of ACN including 0.1% TFA in water(A), 0.1% TFA in ACN(B), Flow rate 1ml/min; 100-90% A in 2min, 90-60% A in 10min, 60-30% A in 2 min, 30-30% A in 3min, 30-100% A in 3 min.

2-1 Preparation of Folate-DOTA derivatives

All procedures were done by using solid phase synthesizer. For the synthesis of FA, Wang Resin which containing protected Lysine with Fmoc and MTT were first used as starting materials. In order to introduce DOTA to the ε position of lysine, MTT was eliminated with 2% TFA in methylene chloride and then reacted with DOTA-mono NHS. After eliminating Fmoc by using 20% piperidine, Pteric acid was introduced using coupling agent. Wang resin was finally eliminated under the condition of TFA:TIS:EDT:Thioanixole:water. The final compound was purified with prep-HPLC and identified by mass spectroscopy(M-1 : 825)

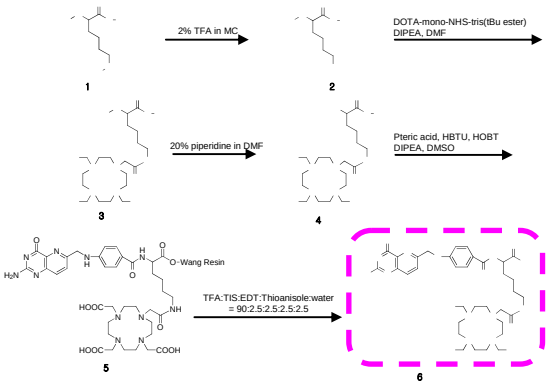

Fig. 2 The synthetic scheme for Folate-DOTA

2-2 Radiolabeling with [^{177}Lu] $LuCl_3$

For radiolabeling of the DOTA folate with [^{177}Lu] $LuCl_3$, a reaction vial of DOTA folate was prepared in 1 M acetate buffer(0.4ml) containing stabilizer (5mg of ascorbic acid and 6mg of dihydroxybenzoic acid). [^{177}Lu] $LuCl_3$ (0.5mCi/ 0.1ml) was added to the reaction vial and incubated at 90 ℃ for 30min. The radio chemical purity was identified by using HPLC. Various labeling parameters such as temperature, ligand concentration and reaction time were performed. As the case of ^{177}Lu, another Lanthanide isotope was used for identifying the labeling efficiency.

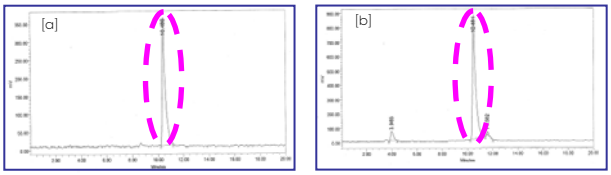

Fig. 3 HPLC data of 177Lu-pteroyl Lys-DOTA(a) & 153Sm-pteroyl Lys-DOTA(b)

2-3 Biodistribution studies

For Biodistribution studies, female normal balb/C mice, aged 7 weeks and 18-23g, have been subcutaneously implanted with the 2×10^6 KB cells and tumors were allowed to grow for 21d. The labeled product (5 uCi / 0.1ml in saline) was injected through the lateral tail vein. At specified time points (2, 24hr), the tumors, blood and various tissues and organs (heart, liver, spleen, kidney, stomach, small intestine, large intestine, lungs) were removed and weighed, and the biodistribution was detected using gamma counter. The results show quick renal clearance. Tumor uptake was shown as 3.313 ± 0.254 %ID/g and 1.525 ± 0.322 %ID/g for 2 and 24 hr, respectively.. The tumor to blood ratio was revealed as 15.1 and 17.15 respectively.

Table 1. Biodistribution (%ID/g)of ^{177}Lu-DOTA-Folate in KB tumor bearing- mice 2 and 24 post injection.

	DOTA		2hr		24hr	
	average	SD	average	SD	average	SD
blood	0.131	0.023	0.193	0.044	0.111	0.021
liver	0.117	0.036	0.484	0.058	0.514	0.063
kidney	1.043	0.016	76.123	6.140	52.377	6.178
spleen	0.174	0.026	0.253	0.043	0.217	0.095
heart	0.234	0.025	0.448	0.100	0.545	0.034
small intestin	0.102	0.042	0.377	0.052	0.179	0.017
large intestin	0.193	0.013	0.876	0.109	0.351	0.039
lung	0.225	0.028	0.566	0.053	0.336	0.032
stomach	0.172	0.040	0.478	0.062	0.243	0.020
tumor	0.107	0.035	3.313	0.254	1.525	0.322

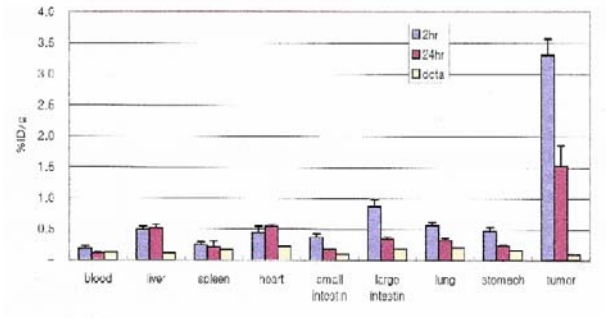

Fig. 4 Biodistribution of ^{177}Lu-DOTA folate in KB cell bearing mice at 2, 24hr after post injection

3. Conclusion

Folate derivatives containing a DOTA system was conveniently prepared through solid phase synthethizer with a 5- step procedure. Radiolabeling procedure was performed by simple incubation under acetate buffer condition to give over 98% yield. The observations derivable form the biodistribution studies with respect to tumor/blood ratio are favorable and therefore folate-DOTA system which is developed our institute is a promising radiotherapeutical agent for tumor treatment.

4. References

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