American Journal of Clinical Medicine Research, 2017, Vol. 5, No. 3, 36-38
Available online at http://pubs.sciepub.com/ajcmr/5/3/3

DOI:10.12691/ajcmr-5-3-3

Biodistribution of Gadolinium-DOTA-PAMAM Dendrimer Generation 3.0-Trastuzumab in Mice Organs

Deasy Biantong, Hari Soekersi*

Department of Radiology, Dr. Hasan Sadikin General Hospital, Universitas Padjadjaran, Bandung, Indonesia
*Corresponding author: hsyabc47@gmail.com

Abstract To know the biodistribution of compounds Gadolinium-DOTA-PAMAM dendrimer generation 3.0-Trastuzumab as a necessary ingredient MRI contrast agents in the organs of mice. Biodistribution test using the compound with radioactive marker 125I is injected intravenously into the blood vessel and distributed to the organs of mice were detected by gamma ray count tool. This research method is a descriptive study. Sampling applied with purposive random sampling technique. Research conducted in the laboratory animal center of Radioisotopes and Radiopharmaceuticals Technology National Nuclear Energy Agency (BATAN PTRR), Serpong, Indonesia. Analysis univariable illustrates the biodistribution of compounds Gadolinium-DOTA-PAMAM dendrimer G 3.0-trastuzumab in the organs of healthy mice regarding percentage per gram organ, bivariable analysis to assess differences in the biodistribution of Gadolinium-DOTA-PAMAM dendrimer G 3.0-Trastuzumab in the organs of mice. Biodistribution differences of Gadolinium-DOTA-PAMAM dendrimer G 3.0-trastuzumab injected intravenously in the organs of mice through percentage per gram organs. Percentage per gram organ compounds injected are highest in the blood, the peak accumulation is at 3 hours and declined in 72 hours, the smallest is in the brain. The liver is the largest organ of elimination for the compound (Gd-DOTA)n-PAMAM dendrimer G3.0-Trastuzumab-125I, peak accumulation is at 3 hours and decreased at 72 hours, then the kidney with the highest accumulated peak at 1 hour and declined to 72 hours.

Keywords: *biodistribution, Gadolinium-DOTA-PAMAM dendrimer G 3.0-Trastuzumab, Magnetic resonance imaging, mice*

Cite This Article: Deasy Biantong, and Hari Soekersi, "Biodistribution of Gadolinium-DOTA-PAMAM Dendrimer Generation 3.0-Trastuzumab in Mice Organs." *American Journal of Clinical Medicine Research*, vol. 5, no. 3 (2017): 36-38. doi: 10.12691/ajcmr-5-3-3.

1. Introduction

Magnetic resonance imaging (MRI) is one of the superior radiological modalities in the medical community, the examination is not invasive and able to assess a detailed anatomy of an organ, especially the soft tissue. Approximately 40-50% of all MRI examination using contrast agents. MRI contrast agents using a gadolinium (III) are typically complexed with ligands form a non-specific complex gadolinium (Gd) [1]. Limitations of MRI examination today, using contrast agents that are not specific and small molecular weight so it's quickly and easily excreted through the kidneys [2].

This research aims to study the biodistribution $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0-Trastuzumab which has been successfully synthesized by Rahmania et al. [2] Contrast candidate compounds consists of $(Gd\text{-}DOTA)_n$ as contrast bound to the polyamidoamine dendimer generation 3.0 (PAMAM dendrimer G 3.0) [3]. PAMAM dendrimer generation 3.0 has 32 pieces of primary amine groups ($-NH_2$) $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0 conjugated to the monoclonal antibody trastuzumab [4].

Biodistribution test $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0-Trastuzumab in this study conducted by marking $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0-Trastuzumab with PAMAM dendrimer radioactive elements with good radiochemical purity [5,6]. $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0-Trastuzumab labeled with radioactive then injected intravenously on several groups of mice (Mus musculus). At a predetermined time mice were sacrificed and the organs were sampled and enumerated by the enumerator gamma rays. Biodistribution $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0-Trastuzumab marked with radioactive as a percentage of injected dose (ID) per gram organ (%ID/g organ) then calculated.

2. Method

The study conducted at the Center for Technology Radioisotopes and Radiopharmaceuticals - National Nuclear Energy Agency (PTRR-BATAN), Serpong, Indonesia, and has received permission from the Research Ethic Committee, Faculty of Medicine, University of Padjadjaran. The subject mice obtained from laboratory Pharmacy Faculty of Padjadjaran University were female gender with an average age of 8-12 weeks and weighed

25-35 grams that have met the inclusion and exclusion criteria.

This research is a descriptive study to assess the biodistribution of $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0-Trastuzumab in the organs of muscle, bone, intestine, blood, kidneys, spleen, liver, heart, lung, stomach and bladder, brain, thyroid of standard trial animals. All reagents were purchased from Sigma-Aldrich, USA.

Sampling with purposive random sampling technique. The samples divided into eight treatment groups, each group consisting of three mice.

Samples were taken with minimal sample quantities based on calculated Federer formula $(r - 1)(t - 1) \geq 15$ (r is the number of samples; t is the number of treatment). Based on the above formula obtained value of $r > 5$ was calculated as follows:

$$(r-1)(8-1) \geq 15; (r-1)(8-1) \geq 15; (r-1)7 \geq 15;$$

$$7r - 7 \geq 15; 7r \geq 22; r \geq 3,1; r = 3.$$

So the minimum sample size for each group are three mice, so the total sample is at least 24 mice.

The collected data is processed in a computerized manner to transform data into information. The steps in data processing starting from editing, coding, data entry, cleaning. Univariable analysis describes biodistribution $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G3.0-Trastuzumab compounds in the organs of mice are presented in percentages per gram organ (%ID/gr organ). Before the bivariable analysis, percentage biodistribution data normality test $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0-Trastuzumab compounds is performed with the Shapiro-Wilks test on a sample size of less than 50 and otherwise normal distribution when the value of $p > 0.05$.

Bivariable analysis to assess differences in the percentage of the biodistribution of compounds $(Gd\text{-}DOTA)_n$ PAMAM dendrimer G 3.0-Trastuzumab by the organs of mice and the time using ANOVA test when the data biodistribution of the compound is normal and Kruskal-Wallis Test if biodistribution the compound is not normal. Data analysis was performed using the SPSS for Windows version 17.0 with the confidence of 95% and p value ≤ 0.05.

Biodistribution test $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab-^{125}I with the injection of 100 μCi (100 μL) $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab-^{125}I administered intravenously in every Group of mice with a body weight of 25-35 grams. At a predetermined time 15 minutes, 30 minutes, hour-1, hour-2, hour-3, hour-24, hour-48, hour-72, and postinjection mice sacrificed. Organs such as blood, bladder, kidney, intestine, stomach, liver, heart, lung, spleen, bone, muscle, brain and thyroid then taken, weighed and enumerated. Percentages per gram organ then calculated.

3. Results and Discussion

A good biodistribution test candidate compounds $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab should use $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G3.0-Trastuzumab marked radioactive ^{153}Gd to avoid introduction of new element / others that may affect the biodistribution of the real $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab. Availability of Gd radioactive (^{153}Gd) is very limited (low specific activity) is not possible to produce $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab ^{153}Gd marked with radioactivity sufficient to test the biodistribution. That is why $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab is labeled with ^{125}I. There are various reasons for selection of ^{125}I as marker for $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab. First, ^{125}I is available with sufficiently high specific activity and free bearers so that $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab marked with ^{125}I can be prepared with sufficient radioactivity for biodistribution test and other tests. Second, the marking $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab with ^{125}I (the protein / trastuzumab) can be done with relative simple procedures. [4] Third, the free iodine (I-) biodistribution is well documented. [6]

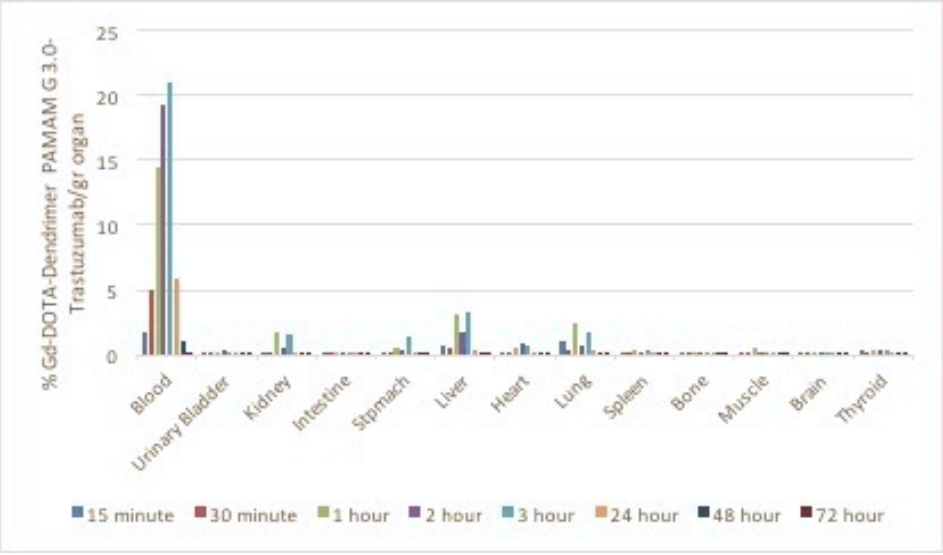

Figure 1. The highest percentage organ contained Gd-DOTA-PAMAM dendrimer generation 3.0-Trastuzumab was in the blood with the highest accumulation at 3 hours and decreased in 72 hours. The second highest percentage organ was in the liver with the highest accumulation score at 3 hours and decreased 72 hours. The third-largest percentage was lungs at 1 hours and decreased 72 hours, then the kidneys with the accumulated time at 1 hours declined in 72 hours. Percentage Gd-DOTA-PAMAM dendrimer generation 3.0-Trastuzumab was found very little in the brain

Marking $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab with ^{125}I give $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab-^{125}I with a radiochemical purity of 93.4%. Radiochemical purity was quite good as a compound to be used in vivo.

Some other radiopharmaceutical even just requiring radiochemical purity > 90% biodistribution compound $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab in the mice organs [7,8].

Trastuzumab-^{125}I is highest in the blood with the highest accumulation, 21.0 ± 3.3%, at 3 hours post-injection and then decreased to 0.1 ± 0.2% at 72 hours. Percentage ID/g organ second highest is at heart of the accumulated value of 3.2 ± 1.1% in 3 hours and decreased to 0.0 ± 0.015% at 72 hours post-injection. Percentage ID/g organ obtained the third highest in the lungs of 2.471 ± 1.4471 hours and down 0.027 ± 0.009% at 72 hours. Meanwhile% ID/g organ in other organs <2%. It can be seen that the% ID/g organ in the kidneys highest 1.688 ± 0.963% at 1 hour and decreased to 0.018 ± 0.004% at 72 hours, % ID/g organ in the stomach highest 1.412 ± 0.465% in 3 hours down to 0.025 ± 0.012% at 72 hours. Percentage ID/g organ at the highest heart 0.125 ± 0.020% 24 hours and decreased to 0.028 ± 0.005% at 72 hours. Percentage ID/g organ $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab smallest was found in the brain. Percentage ID/g organ that is the target organ of thyroid iodine highest 0,186 ± 0,041% at 30 minutes post-injection $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab is also relatively low.

The biodistribution test results of $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab-^{125}I shows % ID/g organ was highest in blood with peak accumulation of 3 hours and continued to decline to 72 hours post-injection. When compared, the % ID / g organ circulation time of contrast agents $Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab-^{125}I in the is blood longer, caused by the molecular weight is relatively larger (~170 kD). Biodistribution test results also showed that elimination organs of dendrimers $Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab-^{125}I are liver and kidneys. The liver is the primary organ of elimination, followed by the kidneys. Percentage ID/g organ $Gd\text{-}DOTA)_n$-PAMAM dendrimer G 3.0-Trastuzumab-^{125}I in the liver and kidneys showed tends to equal to % ID/g organ when compared with the $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G 5.0 as reported by Opina et al. [6,7].

4. Conclusion

Biodistribution test $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G3.0-Trastuzumab-^{125}I which is an analog $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G3.0 has been successful. Percentage per gram organ compounds injected are highest in the blood, the peak accumulation is at 3 hours and declined in 72 hours, the smallest is in the brain. The liver is the largest organ of elimination for the compound $(Gd\text{-}DOTA)_n$-PAMAM dendrimer G3.0-Trastuzumab-^{125}I, peak accumulation is at 3 hours and decreased at 72 hours, then the kidney with the highest accumulated peak at 1 hour and declined to 72 hours.

Conflict of Interest/Funding

None.

Acknowledgements

The authors would like to thank Prof.Hendro Sudjono Yuwono, dr, PhD for helping in preparation of the writing of this paper.

References

[1] Zhou Z, Lu Z-R. Gadolinium-based contrast agents for magnetic resonance cancer imaging. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2013; 5(1): 1-18.

[2] Rahmania H, Mutalib A, Ramli M, Levita J. Synthesis and stability test of radiogadolinium(III)-DOTA-PAMAM G3.0-trastuzumab as SPECT-MRI molecular imaging agent for diagnosis of HER-2 positive breast cancer. *J Radiat Res Appl Sci*. 2015; 8(1): 91-99.

[3] Swanson SD, Kukowska-Latallo JF, Patri AK, Chen C, Ge S, Cao Z, et al. Targeted gadolinium-loaded dendrimer nanoparticles for tumor-specific magnetic renosance contrast enhancement. *Int J Nanomedicine*. 2008; 3(2): 201-210.

[4] Gori S, Montemurro F, Spazzapan S, et al. Retreatment with trastuzumab-based therapy after disease progression following lapatinib in HER2-positive metastatic breast cancer. *Ann Oncol*. 2012; 23(6):1436-1441.

[5] Yavuz B, Pehlivan SB, Unlu N. Dendrimeric systems and their applications in ocular drug delivery. *ScientificWorldJournal*. 2013; 2013: 732340.

[6] Opina AC, Wong KJ, Griffiths GL. Preparation and long-term biodistribution studies of a PAMAM dendrimer G5-Gd-BnDOTA conjugate for lymphatic imaging. *Nanomedicine (Lond)*. 2015; 10(9): 1423-1437.

[7] Vedha Hari BN, Kalaimagal K, Porkodi R, Gajula PK, Ajay JY. Dendrimer: Globular nanostructured materials for drug delivery. *Int J Pharm Tech Res*. 2012; 4(1): 432-451.

[8] Gradishar WJ. Emerging approaches for treating HER2-positive metastatic breast cancer beyond trastuzumab. *Ann Oncol*. 2013; 24(10): 2492-2500.