ORIGINAL ARTICLE AMHSR

Studying a specific tumor marker by a new method and analyzing its efficiency in labeling animals

Mojtaba Navabpour[1] PhD, Zhila Jamshidinia[2] MS, Mohsen Bakhshandeh[1] PhD

[1]*Department of Radiation Technology, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[2]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.*

ABSTRACT

Purpose: To recognize affective factors in delivering X-ray contrast agents (which have high atomic number) via gallium carrier to liver or lymphatic neoplasm cells.
Materials and Methods: Since uptake of gallium by liver has been reported in numerous researches, it was bonded with a contrast media (metrizoate). X-ray imaging was done to determine geometric features of liver.
Results: Radiographic image was obtained. Maximum contrast in radiographic image was observed 72 hours after injection. In addition, increase of effective tumor atomic number caused by metrizoate helped the tumor to be treated by a smart radiotherapy method (i.e. photoelectron therapy). It increased photoelectron therapy efficiency up to two thousand percentages.
Conclusion: A new method of killing cancer cells can be developed using contrast agents. Also, metrizoate-labeled gallium is recommended for marking animals.

Keywords: Pharmaceutical carriers; gallium; metrizoate complex; tumor location; tumor mapping.

AMHSR 2015;13:15-20
www.journals.ajaums.ac.ir

INTRODUCTION

Radiation therapy is the medical use of ionizing radiation to control or kill malignant cells. It has had a very important role in cancer treatment in recent decades. Radiation therapy can be curative if radiation beam is aimed exactly at the tumor. So, in order to reduce normal cell damage during treatment, tumor location must be exactly determined. Unavailability of tumor geometric features leads to less treatment precision and more normal tissue damage, causing unforeseen side effects.[1,2]

Recent researches have shown that drug carriers used in chemotherapy have improved the cancer prognosis up to 45%.[3] So, drug carriers are served to improve delivery and effectiveness of drugs. On the whole, as pharmaceutical complex with high atomic number have significant potential to efficiently detect tumors, Synthesizing them seems reasonable.

So far, gallium has been used as a pharmaceutical carrier in nuclear medicine to deliver radioisotope into the lymphatic tumor to deliver actinomycin (an activator of cancer treatment). Since gallium uptake in neoplasm cells is about 40% more than the other cells, using this complex increased treatment efficiency up to 45%.[4] In this regard, some limitations exist for either material used for treatment or diagnosis. One of them is drug systemic distribution and the permitted dosage. In order to treat specific cells with a drug, distribution must be selective. So extensive studies have been done to find materials that can be distributed selectively with special properties in the body.[5]

Another limitation for drugs administration is accumulation mechanism. When some drugs accumulate on target organ based on its physiological characteristics, it is called physical accumulation. For example, iodine accumulation in thyroid and calcium accumulation in bones. Another accumulation mechanism is active biochemical accumulation. In this case, living units such as antibodies are involved. Accumulation percentage and

effective concentration are other limitations of drug administration. For instance, 80% of iodine accumulates in thyroid gland. Since accumulation percentage is a major issue, obtaining the highest percentage of drug accumulation is considered.[6-11]

Both chemical substances need several properties to be used as a carrier.[12-16] There are some other limitations too. Such as shelf life and effective half-life.[17] Despite of massive efforts, delivery of a specific drug for cancer treatment or drug activators remains a problem that severely restrict the applied lethal dosage for a tumor.

Also, binding X-ray contrast agents with pharmaceutical carriers have not been reported yet, because it was not applied till photoelectron therapy was proposed as a new method for tumor treating. Increasing effective atomic number ratio in target cells compared to the other cells is critical to providing reliable condition for photoelectron therapy or diagnostic purpose.

In this paper, gallium was used as a drug carrier to deliver effective drugs in photoelectron therapy treatment. The proposed complex was a contrast agent containing iodine with atomic number 53 which was bonded with gallium. The goal was to recognize contributory factors in delivering X-ray contrast agents (which have high atomic number) via gallium carrier to liver or lymphatic neoplasm cells.

MATERIALS AND METHODS

The pharmaceutical complex was synthesized and its uptake by liver for treatment via smart radiotherapy method (photoelectron therapy) was studied. Gallium was used as a pharmaceutical carrier which had bonded to metrizoate as an X-ray contrast media with high atomic number. Because of the tendency of gallium in accumulating in the liver, liver was identified as the most suitable organ to be detected in mice.[18-21]

Metrizoate complex and gallium 69 were prepared with 50%, 60% and 75% concentrations in deionized water. Metrizoate complex was injected to mice weighing approximately 150 g in volume of 0.125%. Injections were performed via the mice tail vein and none of the mice were injured. The purpose was to determine the time of maximum accumulation of complex in condition of in vivo. Then radiography was done on the mice 4, 24, 48 and 72 hours after injection. Radiographic density showed the path and accumulation, plus half-life of drug stopping in the animal body with acceptable definition. Since gallium tends to accumulate in the liver, the mice liver was determined as a suitable organ for detecting gallium, especially since their liver was observable in X-ray images regarding its size.

RESULTS

Radiographic images at 4, 24, 48 and 72 hours after injection are shown in **Figures 1** to **4**. The liver can be seen just 4 hours after injection **(Figure 1)**. Radiographic image of rat liver 24 hours after injection is shown in **Figure 2**. Although it was expected to obtain sharper image of the liver in this figure compared to **Figure 1**, unfortunately the image of the liver was disappearing. However, radiographic image of rat liver was followed-up. **Figure 3** shows the radiographic image of rat liver 48 hours after injection. Fortunately, optimum density and also desirable accumulation of gallium in liver, was observed in this figure.

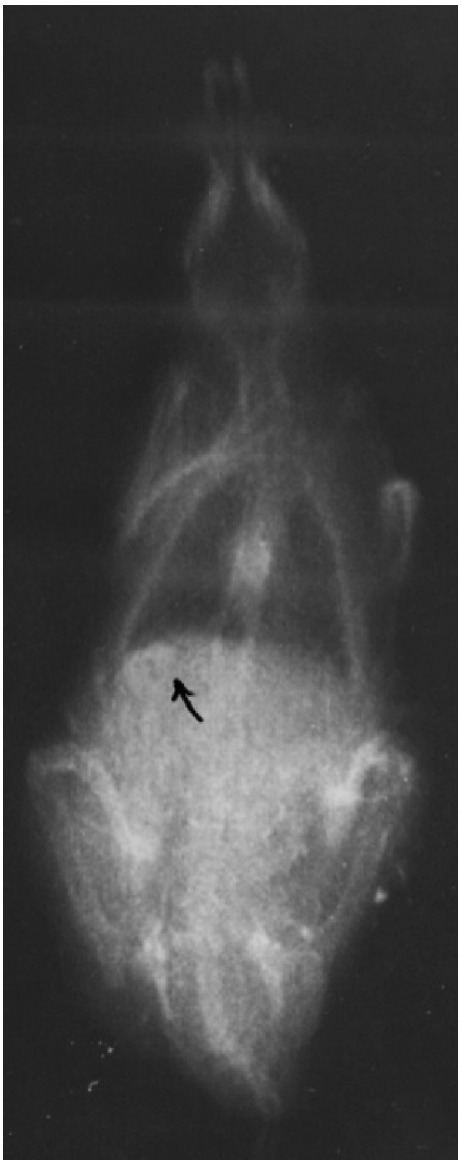

Figure 1. Radiographic image of rat liver 4 hours after injection.

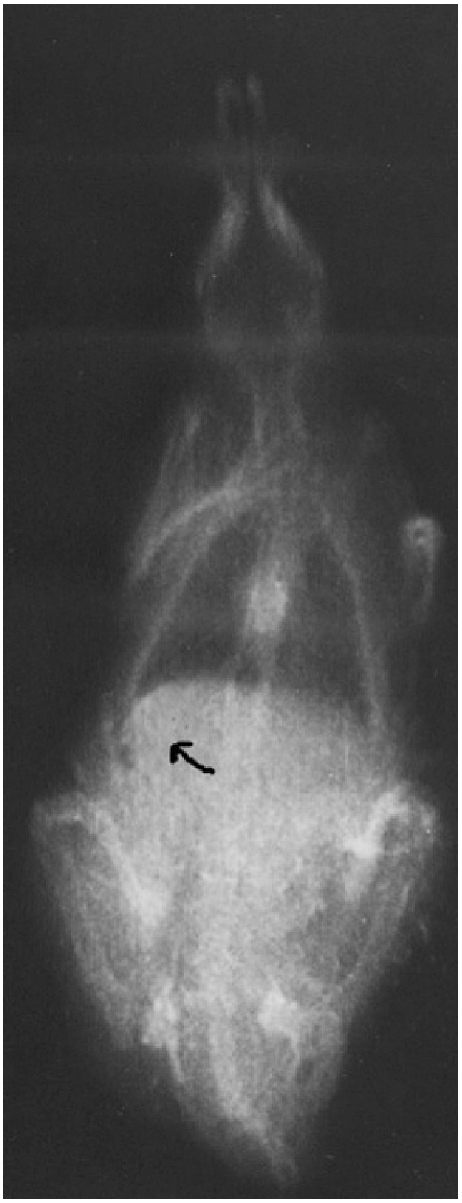

Figure 2. Radiographic image of rat liver 24 hours after injection.

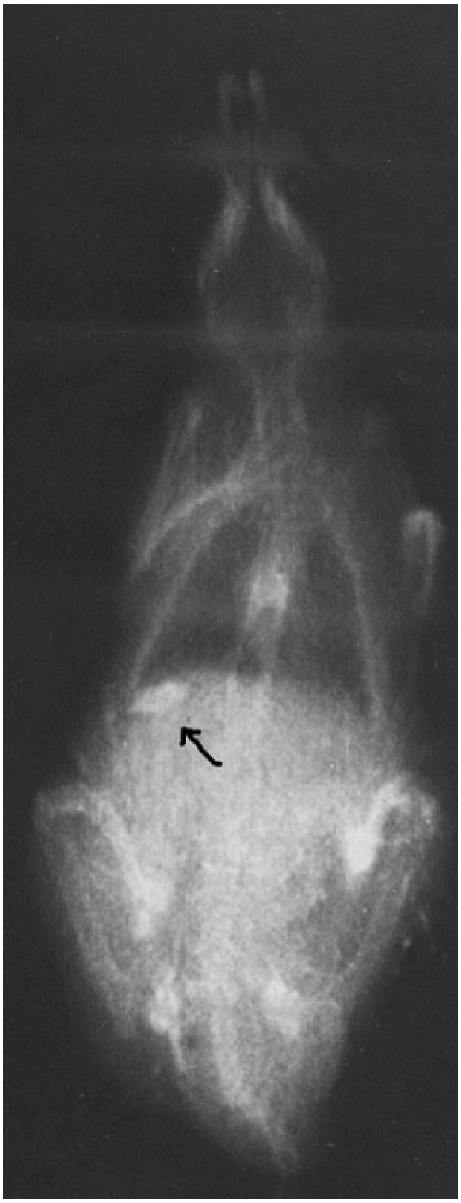

Figure 3. Radiographic image of rat liver 48 hours after injection.

The maximum density difference of mice liver compared to surrounding soft tissues was obtained when radiographic images were prepared from 60 to 72 hours after injection **(Figure 4)**. Since, the comparison of radiographic images may not be accurate enough, optical densitometry was also done. The results confirm radiographic results **(Table 1).** Since radiographic images are negative, lower optical density indicates higher uptake of contrast media. Optical density of mice liver 4, 24, 48 and 72 hours after injection was 0.582, 0.593, 0.514 and 0.431, respectively. Thus, maximum optical density of the liver was observed 72 hours after injection and the minimum 24 hours after injection. The cell experiment results showed that physiochemical properties of metrizoate complex bonded to gallium were similar to gallium alone.

Table 1. Optical density of mice liver.

Number of Figures of Mice	Optical Density
Figure 1. Radiographic image of rat liver 4 hours after injection.	0.582
Figure 2. Radiographic image of rat liver 24 hours after injection.	0.593
Figure 3. Radiographic image of rat liver 48 hours after injection.	0.514
Figure 4. Radiographic image of rat liver 72 hours after injection.	0.431

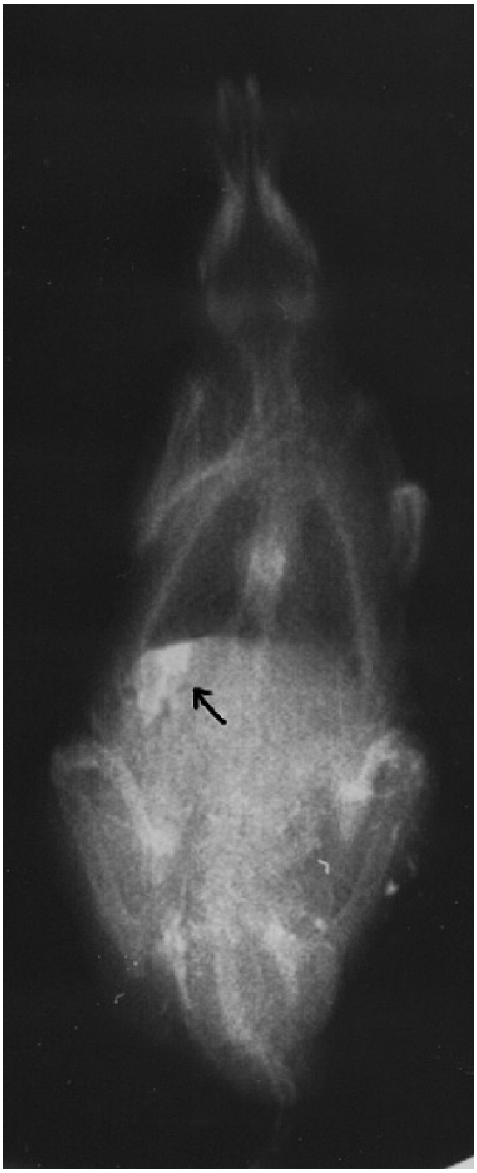

Figure 4. Radiographic image of rat liver 72 hours after injection.

DISCUSSION

Since gallium is the most common carrier used for studying the liver tumors, transmission of metrizoate (as a radiological contrast media) into the liver was studied using gallium as a carrier in the present study.[22,23] The liver can be imaged just four hours after injection, indicating different accumulation of gallium in it. In the other words, the minimum time required for X-ray imaging of the liver was four hours. Although many investigations on the liver are performed by gallium scan, X-ray imaging of liver using gallium has not been reported yet. Since the maximum density difference of mice liver compared to surrounding soft tissues is very important in X-ray imaging, comparing the results of the gallium scan of the liver in the case of minimum time required for X-ray imaging does not make any sense.

Increasing the interval time between injection and X-ray imaging caused a significant decrease in optical density, except in **Figure 2**. Increasing of optical density in **Figure 2** seemed undesirable because of low uptake or uptake pausing. Since uptake was followed for a long time it did not disrupt the results. The maximum density difference of mice liver compared to surrounding soft tissues was 0.165 or approximately 16.5%. It was observed when radiographic images were prepared from 60 to 72 hours after injection **(Figure 4)**. However, in some studies the maximum uptake of gallium was observed 4-12 hours after injection.[24,25] So it may seem that uptake of gallium-metrizoate complex is not as fast as gallium alone. This process may be caused by slow delivery of marker, its excretion from surrounding tissues and remaining in the target cells longer compared to surrounding tissues.

As mentioned above, maximum uptake of metrizoate complex was observed 60-72 hours after injection. This was not because of prolonged uptake of radio generator in liver cells, but due to rapid excreting of complex in surrounding cells compared to liver cells. Actually, it takes maximum four hours to uptake gallium when it is in the vicinity of the cells. Also, radiographic contrast increased due to delay in gallium accumulation in the liver of mice which happened because of different rate of gallium delivery to target organ compared to surrounding tissues. The results showed that different concentrations of metrizoate-gallium complex had no significant effect on its uptake, but more concentrations increase effective atomic number. Also maximum uptake of metrizoate-gallium complex was 15% in liver cells compared to surrounding cells and was observed 72 hours after injection. Thus, effective atomic number of liver cells was 15% more than others. These results showed that using metrizoate-gallium complex is possible in photoelectron therapy which was the goal of this study.

Indeed, as cellular tests showed, uptake time is not more than two hours. But the important point is the difference or ratio of accumulation. If accumulation rate in the tumor and surrounding tissue is 30% and 20% respectively, the ratio will be 1.5. However, if accumulation rate in the tumor and surrounding tissue is 20% and 5% respectively, the mentioned ratio will be 4. Thus, considerable uptake difference will be achieved, which provides desirable implementation of new treatment planning. Gallium accumulation in lymphatic neoplasm cells is nearly 10%

more than the liver cells,[26,27] so measuring accumulation percentage in liver via radiographic density is a suitable index. Also, since the metrizoate-gallium complex can enhance radiologic tissue contrast with increased effective atomic number, the presented study on the metrizoate-gallium complex may be applied in mapping tumors. It also may have applications in the cancer treatment by photoelectron therapy as a smart method.

CONCLUSION

This paper confirms the advantages of metrizoate-gallium complex in increasing photoelectron therapy efficiency up to two thousand percentage, which separates presented study from other researches.

Specific accumulation of X-ray opaque material in neoplasm cells either in form of accumulation on target tissues or accumulation in tumor space leads to exact performance in new radiotherapy treatment methods. As metrizoate-gallium complex uptake in tumor cells increases twenty times (compared to its uptake in normal tissue), all three-dimensional geometrical characteristics for treatment planning and precise calculations can be achieved. In addition, delivering X-ray opaque agents to neoplasm cell increases efficiency of therapeutic method, so the number of treatment sessions can be reduced by half in many cases. Also, the simplicity of the procedure separates it from other techniques such as direct catheterization and since Metrizoate-Gallium complex can be injected, therefore time can be saved. After all, replacement of catheterization as an invasive technique by injection reduces the pain and stress of the patient. Hence, it is expected that selective delivery of X-ray opaque material can be done using other carriers.

REFERENCES

1. Lawrence TS, Ten Haken RK, Giaccia A. Principles of radiation oncology. In: DeVita VT Jr., Lawrence TS, Rosenberg SA, editors. *Cancer: Principles and practice of oncology*. 8th Edition. Philadelphia: Lippincott Williams and Wilkins; 2008:307-27.
2. Svensson GK, Baily NA, Loevinger R, *et al. Physical aspects of quality assurance in radiation therapy.* New York: The American Institute of Physics; 1994.
3. Owunwanne A, Patel M, Sadek S. *The handbook of radiopharmaceuticals*. 1st edition. London: Chapman and Hall Medical; 1994.
4. Woolfenden JM, Carrasquillo JA, Larson SM, *et al*. Acquired immunodeficiency syndrome: Ga-67 citrate imaging. *Radiology.* 1987;162:383-7.
5. Henkin RE, Bova D, Dillehay GL, *et al*. *Nuclear medicine*. New York: Elsevier Health Sciences Division; 2006.
6. Center MS. Mechanisms regulating cell resistance to adriamycin evidence that drug accumulation in resistant cells is modulated by phosphorylation of a plasma membrane glycoprotein. *Biochem Pharmacol*. 1985;34:1471–6.
7. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science.* 2004;303:1818-22.
8. Boura AL, Copp FC, Duncombe WG, *et al.* The selective accumolation of bretylium in sympathetic ganglia and their postganglionic nerves. *Brit J Pharm Chemoth.* 1960;15:265-70.
9. Bray GA. Increased sensitivity of the thyroid in iodine-depleted rats to the goitrogenic effects of thyrotropin. *J Clin Invest*.1968;47:1640–7.
10. Capen CC. Mechanisms of chemical injury of thyroid gland. *Prog Clin Biol Res.* 1994;387:173-91.
11. Alexander WD, Wolff J. Thyroidal iodide transport. *Endocrinology.* 1966;78:581-90.
12. Leong KW, D'Amore PD, Marletta M, *et al.* Bioerodible polyanhydrides as drug-carrier matrices. II. Biocompatibility and chemical reactivity. *J Biomed Mater Res.* 1986;20: 51–64.
13. Lundberg B. Preparation of drug carrier emulsions stabilized with phosphatidylcholine-surfactant mixtures. *J Pharm Sci.* 1994;83:72–5.
14. Braud C, Bunel C, Vert M. Poly (β-malic acid): a new polymeric drug-carrier. *Polym Bull.* 1985;13:293-9.
15. Uekama K, Otagiri M. Cyclodextrins in drug carrier systems. *Crit Rev Ther Drug Carrier Syst.* 1987;3:1-40.
16. Vladimir PT. Drug targeting. *Eur J Pharm Sci.* 2000;11:81-91.
17. Boxenbaum H, Battle M. Effective half-life in clinical pharmacology. *J Clin Pharmacol.* 1995;35:763-6.
18. Mathias CJ, Wang S, Lee RJ, *et al.* Tumor-selective radiopharmaceutical targeting via receptor-mediated endocytosis of Gallium-67-deferoxamine-folate. *J Nucl Med.* 1996;37:1003-8.
19. Lomas FR, Dibos PE, Wagner HN. Increased specificity of liver scanning with the use of 67 gallium citrate. *N Engl J Med.* 1972;286:1323-9.
20. Levin J, Kew MC. Gallium-67-citrate scanning in primary cancer of the liver: diagnostic value in the presence of cirrhosis and relation to alpha-fetoprotein. *J Nucl Med.* 1975;16:949-51.
21. Takeda S, Uchida T, Matsuzawa TA. Comparative study on lysosomal accumulation of Gallium-67 and indium-111 in Morris hepatoma 7316A. *J Nucl Med.* 1977;18:835-9.
22. Aulbert E, Gebhardt A, Schulz E, *et al*. Mechanism of 67Ga accumulation in normal rat liver lysosomes. *Nuklearmedizin*. 1976;15:185-94.
23. James O, Wood EJ, Sherlock S. 67 Gallium scanning in the diagnosis of liver disease. *Gut.* 1974;15:404-10.
24. Scheffel U, Wagner HN Jr, Frazier JM, *et al.* Gallium^{67} uptake by the liver: Studies using ısolated rat hepatocytes and perfused livers. *J NucI Med.* 1984;25:1094-100.

25. Hill JH, Wagner HN Jr. 67 Ga-uptake in the regenerating rat liver. *J Nucl Med.* 1974;15:818-20.

26. Schuhmacher J, Kaul S, Klivényi G, *et al.* Immunoscintigraphy with positron emission tomography: Gallium-68 chelate imaging of breast cancer pretargeted with bispecific anti-MUC1/anti-Ga chelate antibodies. *Cancer Res.* 2001;61:3712-7.

27. Bakshi S, Bender MA. Use of Gallium-67 scanning in the management of lymphoma. *J Surg Oncol.* 1973;5:539-49.

Corresponding Author:
Zhila Jamshidinia, MS
Address: Department of Radiation Technology, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Darband Ave., Qods Sq., Tehran, Iran.
Postal Code: 1971653313
Tel: +98 21 22974598
Fax: +98 21 22974604
Cell Phone: +98 9123236040
E-mail: zhi_jam@yahoo.com

Received at December 2014
Accepted at February 2015