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Assessment of metrezoate-labeled gallium transmission in cultured human Burkitt lymphatic cells

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Background: In addition to determining the exact tumor location and its geometric features, an increase of the effective tumor atomic number will enhances the chance in treating the tumoral cells under suitable radiation. In the present study, we assessed metrezoate-labeled gallium transmission in Burkett lymphatic cells. ***Materials and Methods:*** Human Burkitt lymphatic cells were grown in culture media. Metrezoate-76% labeled with gallium-67 and the developed complex was used with a volumetric amount of 125%. The complex was added to the culture media, and then the absorbed volume was determined. Through the SPECT imaging system, the culture media containing the lymphatic cell colonies were scanned 1, 2, 4, 8, 24, 48, and 72-hr post-addition of metrezoate-gallium complex. The transmitted activities in the colonies area were measured after imaging. ***Results:*** Gallium-metrezoate complex is significantly accumulated in malignant lymphatic cells. The study of the results throughout 72-hr revealed that most absorption, count quantity and transmitted activity had all occurred 4-hr after adding gallium-metrezoate complex solution. ***Conclusion:*** It can be concluded that gallium-metrezoate complex can be significantly accumulated in the Burkett lymphatic cells and uptake is non-linear with exposures time. This method of enhancing effective atomic number in malignant lymphatic cells therefore appears promising. **Iran. J. Radiat. Res., 2012; 10(2): 99-104**

Keywords: *Effective atomic number, lymphatic tumoral cells, gallium-metrezoate.*

INTRODUCTION

Selective transmission of the medicinal elements to target tissues and organs for specific diagnostic tests and treatments is among the important goals in medical studies. For example, in numerous studies in nuclear medicine the transmission of radiopharmaceutical to the target organ is performed with links to chemical kits, monoclonal antibodies [1-5], or transmission of radionuclides to malignant cells by vehicles such as Vitamin B12, bleomycin, or actinomycin, which are used in chemotherapy [6,7].

Gallium-67 in citrate form is the most common radioisotope used for the localization of the tumors, so that the gallium scanning has been widely evaluated for imaging of the lung carcinoma, hepatomas, and lymphomas. Though the exact localizing mechanism of gallium in neoplastic cells is unclear, it has become evident that gallium transmission into the cells is associated with lysosomes functions [8-10].

In the present study, transmission of metrezoate, which is radiological contrast media containing iodine with the atomic number 53, into the lymphatic cancer cells was studied utilizing gallium-67 as a medicinal vehicle. The results obtained may enhance radiologic tissue contrast with increased effective atomic number in localizing tumors; and/or may have applications in the cancer cell absorptive dose and treatment output under suitable radiation.

MATERIALS AND METHODS

The in-vitro studies and imaging

Gallium was used to bond with

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metrezoate, an iodinated contrast media, to produce gallium-metrezoate complex. It should be borne in mind that in this complex, metrezoate possesses a high atomic number and the transmission of this complex into the cells increases the effective atomic number of malignant cells with a lymphatic origin [8-10]. In this study the complex of gallium-67 citrate and Metrezoate-76% was developed in water and by considering the recommended dose of the x-ray contrast element, the developed complex was used with a volumetric amount of 125% (1.8 cc).

Malignant lymphatic cells (Human Burkitt Lymphoma, Daldi, NCBI Code 112, Pasteur Institute, Tehran, Iran) were obtained from the National Cell Bank of Iran (NCBI). Cells were grown in alpha minimum essential medium (αMEM, Sigma) supplemented with 15% fetal bovine serum (FBS, Gibco, BRL), 1% L-glutamine and antibiotics (Penicillin 100 IU/ml, Streptomycin 100 µg/ml, Sigma). Cells were then routinely grown in 25-cm^2 flasks, and incubated at 37°C in a 90% humidified atmosphere of 10% CO2 in air as normoxic condition. In order to repeat the tests in similar conditions and calculate mean±SD of the measurements, exponentially growing cells were sub-cultured in 10 glass flasks under the normoxic conditions and treatments. Approximately after 95 hours, 1cc of gallium-metrezoate complex in physiological serum solution with an activity of 50 µCi was added to each of the culture medias so that it was distributed on a medium surface. In order to determine the *in-vitro* accumulation of the complex, the absorbed volumes in cell colonies were calculated at 1, 2, 4, 8, 24, 48, and 72-hr intervals. This volume includes the primary volume minus the sum of the remaining volume and the absorbed volume in background of the culture medium.

Gallium-67 in gallium-metrezoate complex is a gamma emitter with energies of 93 keV (40%), 184 keV (24%), 296 keV (22%) and 388 keV (7%) which permits the imaging through SPECT (Single Photon Emission Computer Tomography) scanning method [11]. In this study, planar imaging were acquired using a single head SPECT system (Ecam Siemens Medical Solutions, USA, Inc) with medium energy general purpose (MEGP) collimator and the system full width at half maximum (FWHM) of 9.4 mm. The planar imaging using a full field of view was 128×128 with a pixel size of 4.42 mm. Each of the three major photon energies emitted by Ga-67 had a centered 20% photopeak window and three photopeak windows were summed at the time of acquisition. Gallium scan imaging of each culture media was conducted in 5 minutes at 1, 2, 4, 8, 24, 48, and 72 hrs post-addition of gallium-metrezoate complex solution to the medias and finally the activity count, transmitted activity (λ=0.0086 hr^{-1}, Detector geometry=2π) and area of the transmitted activity in colonies were calculated using the acquired images. Since the colonies have a thickness less than or equal to the pixel size imaging, so at the level of the colonies, counts per pixel in the colony's volume is determined.

Statistical analysis

All data are expressed as mean± standard deviation (SD) and then comparison of differences was done with one-way ANOVA. Results were considered significant when the probability value was <0.05. All of the statistical analyses were performed using the SPSS13 software package.

RESULTS AND DISCUSSION

Chemical analysis revealed that the metrezoate contrast media would produce a suitable and stable compound with gallium citrate; therefore, gallium was considered useful in gallium-metrezoate complex. The generated bond was a 1:1 ionic bond; consequently, all gallium molecules were engaged with metrezoate molecules, and this process maximized the transmission. Figure 1 depicts gallium-metrezoate complex as the

specific marker used for malignant tumors with lymphatic origin.

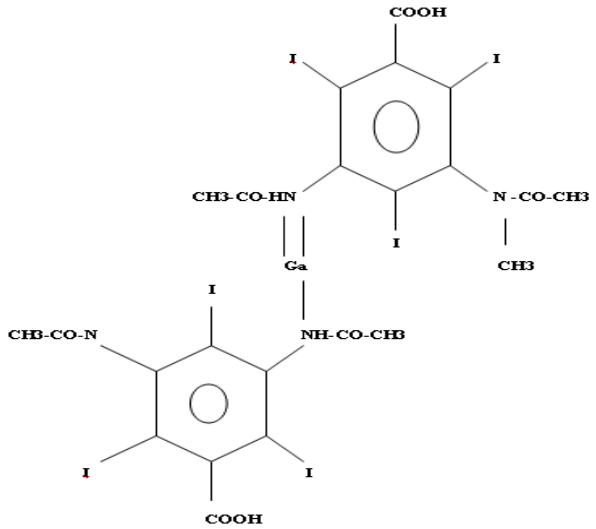

Figure 1. The extended gallium-metrezoate compound.

Figure 2 shows the absorbed volume of gallium-metrezoate complex by lymphatic cells at different times. The results showed that maximum absorption occurring 4-hr post-addition of the complex to the culture media, though the statistical analyses revealed no significant differences in the groups under study ($p > 0.05$). The comparison between the absorbed volume in the cells and the culture media background following 4-hr demonstrated that around 90% of the absorption had occurred in lymphatic cells; thus, the average effective atomic number in lymphatic cells had been 9 times greater than that of the culture media background.

The observation of the gallium-67 scanning images revealed that the accumulation of the gallium-metrezoate complex in cell colonies 4-hr post-addition of the complex solution to the culture media would be maximized (figure 3).

The statistical analysis of variance demonstrated that the count (36250±3987, $p<0.05$), transmitted activity (15.7±1.7 µCi, $p<0.05$) and the mean area of the colonies (25.8 ± 3.8 mm², $p<0.05$) predicts a significant difference at 4-hr following the addition of the complex solution to the culture media at 1, 2, 8, 24, 48, and 72-hr (figure 4).

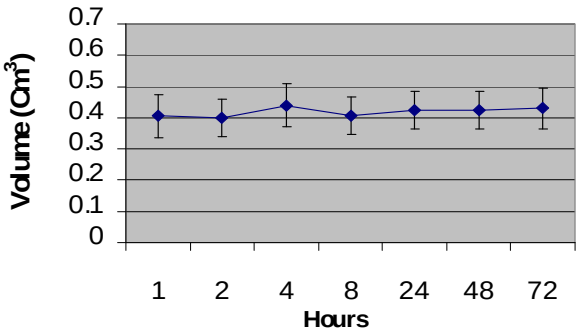

Figure 2. The amount of gallium-metrezoate complex absorbed volume in malignant lymphatic cells in culture media at different measured time intervals

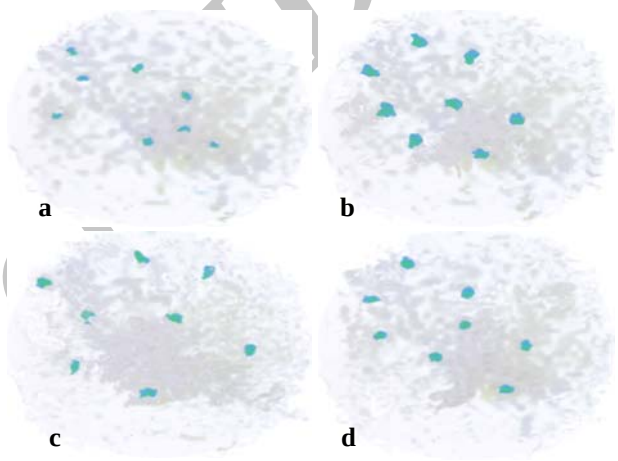

Figure 3. Gamma scan images of the malignant lymphatic cell culture media at different time intervals following addition of gallium-metrezoate complex solution. Gallium present in the complex is the Gamma radiating element. Pictures a to d are respectively obtained at 1, 4, 24, and 48-hr after adding the complex solution. The images show that there have been 8 colonies reproduced in culture media.

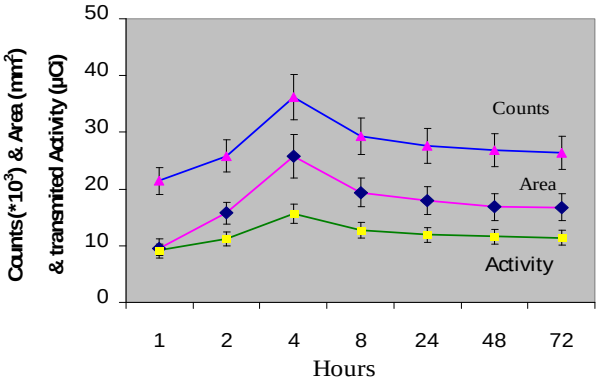

Figure 4. The results (mean±SD) of the counts, transmitted activities and colony areas in the Gamma scan images of the malignant lymphatic cell culture media at 1, 2, 4, 8, 24, 48, and 72-hr after adding gallium-metrezoate complex solution to the culture media.

CONCLUSION

In the present study, gallium-metrezoate complex was developed and used as the vehicle for transmitting metrezoate into the human Burkitt cells. So far, it was shown that Gallium has excessively been absorbed in neoplastic cells, especially those with lymphatic origin [12-17]. Consequently, in our study, gallium was used as a means to transmit metrezoate into the lymphatic cells, and the results indicated that gallium-metrezoate complex significantly accumulated in malignant lymphatic cells. The uptakes of gallium-67 into different cell lines are known to be linear or rose steeply toward an eventual plateau with exposures time [18]. Unlike the gallium, transferrin-stimulated ^{59}Fe uptake was maximum for 24-hr exposures and no increase was detected with 48-hr exposures and uptake was non-linear [18, 19]. Our results indicates that (figures 2 and 4) gallium-metrezoate uptake in colonies of the malignant lymphatic cell culture media is non-linear and uptake is maximum for 4-hr exposures and no increase was detected with 8, 24, 48, and 72-hr exposures. Chitambar *et al.* showed that transferrin receptor-dependent and transferring-independent mechanisms exist for the uptake of ^{67}Ga in cells [20, 21]. Transferrin has been shown to form stable complexes with gallium and it would appear logical to assume that the cellular uptake is mediated by cellular transferrin receptors [22, 23] and the action of transferrin is a critical feature of tumors' cellular uptake [24-26].

The aim of high precision conformal radiotherapy and other high precision radiotherapy techniques such as stereotactic radiotherapy [27] and photoelectron therapy [28] is to achieve the highest dose differential between tumor and normal tissue by potential increase in tumor dose without increasing normal tissue damage [29]. We hypothesized that metrezoate labeled by gallium can be used to increase effective atomic number in malignant lymphatic cells. Our results showed a significant absorption in these cells and, therefore, the effective atomic number increased compared with the number observed in the culture media background. It can be, therefore, expected that in the presence of ionizing radiations, a significant energy enhancement will be observed in the target cells in proportion to the increased atomic number.

Joubert *et al.* made *in-vitro* measurements on Bovine Aortic Endothelial Cells survival and *in-vivo* experiments on Fisher rats bearing F98-glioma for assessing the dose enhancement factor (DEF) according to the iodine concentration. The observed DEF for the endothelial cells was 2.7 for an iodine concentration of 10 mg/ml. In addition, the *in-vivo* experiment showed a significant increase of survival time when the rats were irradiated in the presence of iodine [30]. Physical dose enhancement that calculated accurately by Monte Carlo method, described by Boudou and Rousseau [31]. These researchers found that the secondary particles such as characteristic X-rays, photoelectrons and Auger electrons generates at a higher yield by the medium energy X-rays on the high-atomic number atoms. Finally, they reported a localized dose enhancement in the tumor cells [32, 33].

The succession in the time of a high iodine concentration in the tumor cells, give the opportunity to better treatment with the augmentation of radiation effect on tumor tissue [30].

Throughout 72-hr, our results revealed that when gallium-metrezoate complex was in the vicinity of the lymphatic cells, the most absorption had occurred after 4-hr and this delay could probably result from the physiological process of transmission of the complex to the target cells. Though the average absorption of 4-hr did not provide any significant statistical differences, the count quantity, transmitted activity and area demonstrated a statistically significant difference which may be due to the high precision of the SPECT method in measurements.

Up to the best of our knowledge, the

development of a medicinal complex with the X-ray contrast elements on malignant lymphatic cells has been the first time happening in this study.

More investigations using other malignant cells, *in-vivo* experiments, preparation of the grounds for the most suitable radiations and absorption doses are our study limitations. If the gallium-metrezoate enhances tissue contrast in localizing tumors, may have applications to identify the metastatic cancers accurately, and/or may consequently augments radiotherapy or stereotactic radiation therapy to increase the dose deposition accuracy and precision of target localization, thereby reducing the amount of healthy tissue in the treatment field.

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