^{68}Ga-Glutathione radio-complex as a new diagnostic probe for targeting colon cancer in rats

Sidiq Sumeera[1] MSc, Devinder K Dhawan[2] MSc, PhD, Ravi Ranjan Kumar[1] MSc, Jaya Shukla[3] MSc, PhD, Bhagwant R Mittal[3] MBBS, MD, Vijayta D Chadha[1] MSc, PhD

1. Centre for Nuclear Medicine (UIEAST), Panjab University and 2. Department of Biophysics, Panjab University and 3. Department of Nuclear Medicine, Post Graduate Institute for Medical Education and Research, Chandigarh, India

Keywords: ^{68}Ga-Glutathione - Colon cancer - Rats with colon cancer

Corresponding author:
Dr. Vijayta D Chadha,
Centre for Nuclear Medicine,
Panjab University,
Chandigarh-160014, India
Tel.: 91-01722534141
vdchadha@pu.ac.in

Received:
2 May 2019
Accepted revised:
31 May 2019

Abstract

Objective: Glutathione (GSH) plays an important role in a horde of cellular events that include cell proliferation and apoptosis. The present study describes the radiosynthesis and characterization of gallium-68 (^{68}Ga)-labelled glutathione for its application in radionuclide imaging of cancer. **Animals and Methods:** The radiosynthesis of radio-complex ^{68}Ga-GSH was performed by the direct labeling method. The developed radio-complex was subjected to quality control tests. Colon tumors were developed in healthy male Sprague Dawley (S.D) rats by giving subcutaneous injections of dimethylhydrazine (DMH) in order to monitor the uptake of ^{68}Ga-GSH radio-complex. **Results:** Gallium-68-labelled glutathione was synthesized with a labeling efficiency of 73.5%±1%. Percentage plasma protein binding and log Po/w values for the radio-complex were found to be 20%-30% and -0.223±0.12, respectively. A significantly higher percentage specific uptake value of newly developed ^{68}Ga-GSH complex was observed in colon tumor in comparison to soft tissue at 90 minutes post administration thereby exhibiting specificity for cancerous cells, which was also witnessed significantly increased overtime from the ratio of colon tumor uptake to normal colon uptake ($P \leq 0.05$). **Conclusion:** Therefore, ^{68}Ga-labelled glutathione can further be exploited for radionuclide imaging and assessment of tumor drug resistance in patients.

Hell J Nucl Med 2019; 22(2): 131-134 *Epub ahead of print: 7 July 2019* *Published online: 20 July 2019*

Introduction

Glutathione (GSH), a water soluble tripeptide, is the most abundant intracellular low molecular weight thiol [1]. It is synthesized de novo from the amino acids glutamic acid, cysteine and glycine. Most of the cellular GSH (85%-90%) is present in cytosol with remainder in many organelles [2]. Glutathione is found in millimolar concentration in most tissues with an intracellular concentration of 1-10mM in mammalian cells and only amicromolar concentration of GSH is found in plasma [3]. Glutathione plays a fundamental role in detoxification of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and also regulates the intracellular redox environment [4]. Changes in GSH homeostasis have been implicated in the etiology and progression of a variety of human diseases like cancers, neurodegenerative disorders and aging [5, 6]. Further, polymorphic expression of enzymes involved in GSH homeostasis influences susceptibility and progression of disease pathologies [7].

Elevated GSH levels have been demonstrated in colorectal, lung, breast, ovarian, head-neck and hematologic malignancies [8, 9]. Many cancer cells including lung cancer have shown up regulation of GSH metabolism and also cancer cell lines made resistant in vitro were demonstrated to have higher GSH contents [10]. Therefore, assessing the tumor GSH levels with the aid of molecular imaging can be exploited for its role in disease diagnosis and timely assessment of therapeutic response. In an earlier study, Wongso et al. (2013) have successfully radiolabeled GSH with radionuclide ^{99m}Tc thereby indicating its cancer targeting potential [11].

Nuclear medicine imaging is an emerging and potentially revolutionary discipline that aims to visually characterize normal and pathologic processes at the cellular and molecular levels. Positron emission tomography (PET) and single photon emission tomography (SPET) are being routinely used in clinical practices as such [12]. These imaging modalities remotely sense the cellular and molecular events by detecting radioactive emissions from the localized radiopharmaceuticals. Fluorine-18-fluorodeoxyglucose (^{18}F-FDG) PET is used fordiagnostic, follow-up and therapeutic evaluation of patients with colorectal and other cancers. However, its use for early detection of primary colorectal

cancer is limited due to its low sensitivity for small tumors. Patients with fistulas, inflammatory bowel disease, abscesses, diverticulitis can cause false-positive findings [13]. Also, the nuclear radiopharmacy for ^{18}F-FDG synthesis is cyclotron dependent and involves complicated radiolabeling chemistry with longer synthesis time. Other radiopharmaceuticals have also been evaluated in colorectal cancer patients which include ^{18}F-fluoro-L-thymidine, technetium-99m-anticancero-embryonic monoclonal antibody (^{99m}Tc-anti-CEA) Fab8 fragment and ^{99m}Tc-bombesin. However, these were found to be less efficient than ^{18}F-FDG [14]. On the other hand, ^{68}Ga-a positron emitting radionuclide that does not require cyclotron for its production, is a suitable candidate for radiolabeling in routine clinical set-up [15]. It has easy labeling chemistry with a physical half-life of 67.71 minutes and is compatible with the pharmacokinetics of several radiopharmaceuticals with low molecular weights. Due to its short half-life, delivers minimum radiation dose to the patient and personnel and also provides sufficient levels of radioactivity for high quality images. Therefore, keeping into consideration the advantages of ^{68}Ga with its higher sensitivity and higher resolution, we for the first time propose to label it with glutathione in order to develop it as a dimer for localizing colon tumors. We optimized the radiosynthesis of radio-complex ^{68}Ga-GSH and bio-evaluated it as a radionuclide probe in imaging of experimental colon carcinogenesis.

All laboratory materials used for this study were purchased from India. The radio-synthesis of ^{68}Ga-GSH was performed by the direct labeling method and the percentage labeling efficiency was carried out by ascending chromatographic technique. The pH of the eluate $^{68}GaCl_3$ obtained from the ^{68}Ge/^{68}Ga generator was adjusted to 5-5.5 using 0.5M sodium acetate. Optimization of reaction time, chemical constituents such as reduced GSH as well as the stability of the resultant complex with pH and time, was investigated in order to maximize the radiochemical yield. After adjusting the pH (5-5.5), variable amount (20-140μg) of reduced GSH was added to $^{68}GaCl_3$ (74MBq) solution and then kept at ambient temperature for 30min to achieve maximum labeling efficiency. The maximum yield of 73.5±1% was achieved with 60μg of glutathione.

Each factor in the experimental studies was repeated three times and differences in the data were evaluated with student's t-test. Results were reported as mean±standard deviations (SD). The level of significance was set at $P \leq 0.05$.

Paper electrophoresis was performed to determine the net charge on ^{68}Ga-GSH radio-complex and to separate different radiochemical species, followed by mass spectrometry. The paper electrophoresis results indicated that the ^{68}Ga-GSH migrated with different mobility and also indicated the anionic nature of ^{68}Ga-GSH. Further, the m/zof ^{68}Ga-GSH was calculated at 679.22 and from the mass spectroscopy results, it was evident that the GSH-^{68}Ga-GSH complex is formed with molecular mass of around 682.64.

We also verified the stability of the radio-complex in serum through blood samples drawn from rats (subjected to light ether anesthesia) by puncturing the retro-orbital plexus using sterilized glass capillaries and were kept for 2hrs at room temperature. Protein binding of the radio-complex was measured and expressed as a fraction of radioactivity bound to protein to the total activity. The ^{68}Ga-GSH was observed to be stable for 2hrs at room temperature in serum. The protein binding of ^{68}Ga-GSH assessed in plasma was found to be 20%-30%.

Lipophilicity is one of the parameters of drug/substance which influence its biological activity. The logP values are used as the measure of lipophilicity. The partition coefficient (Po/w) was calculated as the ratio (activity in the n-octanol layer)/(activity in the aqueous layer) and was expressed as log Po/w. Log Po/w value for ^{68}Ga-GSH was found to be -0.223± 0.12, indicating that ^{68}Ga-GSH is hydrophilic in nature. Since water solubility of a radiopharmaceutical prevents its precipitation at physiological pH in the blood, this property of ^{68}Ga-GSH would allow it to target small organs having low blood supply without forming insoluble aggregates while in circulation. Water solubility would also facilitate easy excretion through kidneys thus reducing radiation dose to target organs.

From the blood pharmacokinetic studies, radio-complex ^{68}Ga-GSH was observed to follow biphasic clearance pattern. The first clearance phase was observed within 45min of intravenous (i.v.) administration followed by second clearance pha-

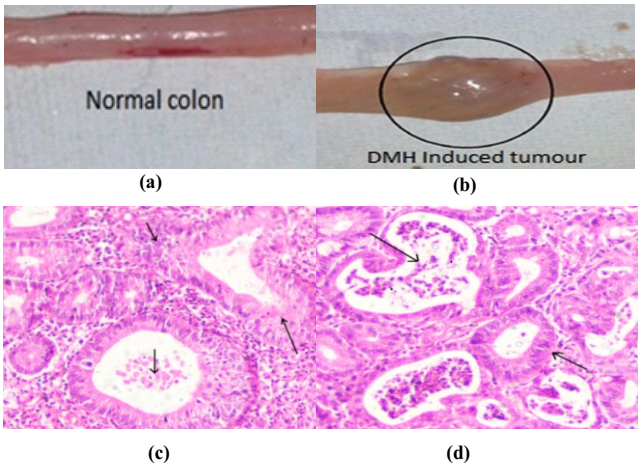

Figure 1. a) Normal morphology of control colon. b) Morphological changes in the DMH treated colon. c,d) Microscopic images of transverse sections of DMH treated colon (stained with H/E stain) reveal enlarged nuclei, thickening of epithelium, hyper-chromatic cells and increased mitotic activity.

se at 90min post administration.

Twelve rats were used as normal controls. Another twelve rats received dimethylhydrazine for fourteen weeks at a dose level of 30mg/kg body weight to induce colon cancer [16]. Tissue sections of control animals displayed normal colonic histoarchitecture with no apparent signs of abnormality. However, dysplasia and well differentiated signs of adenocarcinoma were evident following DMH treatment (Figures 1c and 1d). Histological investigations revealed enlarged nuclei, thickening of epithelium, hyper-chromatic cells and increased mitotic activity.

Bio-distribution of ^{68}Ga-GSH radio-complex was evaluated in different organs after the i.v administration through penile vein, in rats. The highest uptake was witnessed in bladder followed by kidneys, liver in control and DMH treated rats shown in Tables 1and 2. The highest bladder activity was observed at 45min. Further, the small intestine and the large intestine showed a significant percentage uptake post injection which was found to be increased significantly as a function of time up to 45min and then followed by a decrease in activity at 90min. The most significant finding of the study was a high uptake at the site of tumors with maximum uptake witnessed at 90min ($P \leq 0.05$) when compared to uptake in the colon of control rats. The study revealed that the ratio of colon tumor uptake to normal colon uptake increased with time in DMH treated rats and tumor to muscle ratio increased with time attaining maximum value at 90min after i.v administration.

Table 1. *Biodistribution of ^{68}Ga-GSH in normal rats.*

Organ	15min	45min	75min	90min
Colon	1.726± 0.345	2.740± 0.363	0.640± 0.087	0.536± 0.106
Heart	1.340± 0.650	1.230± 0.196	0.267± 0.083	0.420± 0.036
Spleen	1.583± 0.338	1.673± 0.135	0.760± 0.158	0.586± 0.249
Liver	2.05± 0.643	1.640± 0.474	0.690± 0.207	0.560± 0.168
Kidney	2.326± 0.691	1.933± 0.162	0.796± 0.128	0.743± 0.075
Lung	1.99± 0.318	1.88± 0.245	0.630± 0.199	0.55± 0.025
Small intestine	1.3700± 0.115	1.700± 0.062	0.816± 0.271	0.616± 0.075
Bladder	4.440± 0.449	7.006± 1.178	4.303± 1.913	2.373± 0.736
Brain	0.160± 0.026	0.146± 0.023	0.060± 0.010	0.046± 0.005
Prostate	1.010± 0.264	1.373± 0.374	0.216± 0.058	0.203± 0.064
Bone	0.305± 0.251	0.853± 0.149	0.226± 0.065	0.170± 0.052
Muscle	0.570± 0.190	0.500± 0.079	0.213± 0.153	0.070± 0.034

All values are expressed as Mean ± SD; n=3

Table 2. *Biodistribution of ^{68}Ga-GSH in DMH treated rats.*

Organ	15min	45min	75min	90min
Colon	0.850± 0.593	1.670± 0.430	1.246± 0.098	1.066± 0.159
Colon tumor	1.443± 0.359	2.820± 0.590	3.073± 0.500	3.367± 1.017
Heart	1.330± 0.599	1.060± 0.305	0.950± 0.045	1.140± 0.190
Spleen	1.816± 0.351	2.263± 0.457	1.560± 0.181	1.470± 0.320
Liver	1.876± 0.339	1.693± 0.383	1.326± 0.522	1.303± 0.638
Kidney	2.376± 0.616	1.490± 0.138	1.266± 0.065	1.340± 0.134
Lung	1.450± 0.409	1.350± 0.225	1.296± 0.165	1.183± 0.155
Small intestine	0.830± 0.232	1.280± 0.036	1.286± 0.080	1.256± 0.115
Bladder	3.234± 0.948	5.703± 2.718	3.3467± 1.671	2.330± 0.802
Brain	0.223± 0.020	0.243± 0.035	0.230± 0.755	0.200± 0.026
Prostate	0.503± 0.153	0.856± 0.200	0.773± 0.260	0.583± 0.192
Bone	0.790± 0.295	0.790± 0.234	0.763± 0.051	0.6833± 0.361
Muscle	0.710± 0.190	0.626± 0.162	0.453± 0.066	0.370± 0.147

All values are expressed as Mean ± SD; n=3

The maximum tumor uptake was obtained at 90min post i.v administration. As from the blood pharmacokinetic pattern, ^{68}Ga-GSH radio-complex was observed to follow biphasic clearance pattern with a second peak at 90min, which may be due to release of ^{68}Ga-GSH from various organs back into systemic circulation. This can be corroborated with the maximum tumor uptake at 90min post i.v administration, providing systemic supply of ^{68}Ga-GSH to the tumor site and resulting in maximum uptake. The increased pattern of uptake with time in tumors indicated the retention of ^{68}Ga-GSH radio-complex in DMH induced colon tumors. Increased uptake of ^{68}Ga-GSH could be linked with the increased expression of gamma-glutamyltransferase (GGT), which is a

key enzyme involved in glutathione metabolism and is reportedly increased in malignant cells. However, further studies are warranted to assertively corroborate our finding with enhanced levels of GGT in experimental model of colon tumors. Further, oxidative stress has long been implicated in cancer development and progression, resulting in generation of enhanced levels of reactive oxygen species, free radicals and electrophiles that lead to increase demand for GSH resulting in increased ^{68}Ga-GSH uptake.

The present findings convincingly provide evidence of ^{68}Ga-GSH specific accumulation at the site of colon cancer tumor in rat. However, its validation as a feasible option in clinical practice needs further exploration both in cell lines and preclinical models to correlate its sensitivity and accuracy with GGT over expression for scintigraphic visualization. Owing to substantial evidences that GSH plays an essential role in cancer progression and drug resistance in several cancers, the radio-complex ^{68}Ga-GSH may allow the noninvasive evaluation of the GGT over expression in cancerous tissue and may be exploited as a probe for the assessment of the drug resistance in tumors. Further, our findings with ^{68}Ga-GSH radio-complex also provide a reason to exploit GGT as a therapeutic target by suitably labeling GSH with therapy based radionuclides currently used in clinical practice.

The authors declare that they have no conflicts of interest.

Acknowledgements
The financial support by the Department of Science and Technology (DST-PURSE), New Delhi is gratefully acknowledged.

Bibliography

1. Angel LO, Salvador M, Jose ME. Glutathione in Cancer Cell Death. *Cancers* 2011; 3: 1285-310.
2. Kifayatullah S, Muhammad FK, Amir B. Effect of cisplatin on glutathione redox status in isolated plasma and cytosolic fraction 2013. *Afr J Pharm Pharmacol* 2013; 7: 37-45.
3. Davide M, Christine T, Jakob RW et al. Intracellular glutathione pools are heterogeneously concentrated. *Redox Biol* 2013; 1: 508-13.
4. Nicola T, Roberta R, Mariapaola N et al. Role of Glutathione in Cancer Progression and Chemoresistance. *Oxid Med Cell Longev* 2013; 972913:10.
5. Ankita B, Celeste MS. Glutathione metabolism in cancer progression and treatment resistance. *J Cell Biol* 2018; 217: 2291-8.
6. William MJ, Amy LW, John JM. Dysregulation of Glutathione Homeostasis in Neurodegenerative Diseases. *Nutrients* 2012; 4:1399-440.
7. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother* 2003; 57(3): 145-55.
8. Michael PG, Mohit SK, Stephanie DT et al. Glutathione levels in human tumors. *Biomarkers* 2012; 17(8): 671-91.
9. Ping Y, Ebbert JO, Zhifu S et al. Role of the Glutathione Metabolic Pathway in Lung Cancer Treatment and Prognosis: A Review. *J Clin Oncol* 2006; 24(11): 1761-9.
10. Melissa AF, Iman MA, Carmen JS et al. Enhancement of carboplatin-mediated lung cancer cell killing by simultaneous disruption of glutathione and thioredoxin metabolism. *Clin Cancer Res* 2011; 17: 6206-17.
11. Wongso H, Zainuddin N, Iswahyudi I. Biodistribution and Imaging of the ^{99m}Tc-Glutathione Radiopharmaceutical in White Rats Induced with Cancer. *Atom Indonesia* 2013;39(3): 106-11.
12. Adak S, Bhalla R, Vijaya Raj KK et al. Radiotracers for SPECT imaging: current scenario and future prospects. *Radiochim Acta* 2012;100: 95-107.
13. Ettore P, Desiree D, Laura C et al. Diagnostic Applications of Nuclear Medicine: Colorectal Cancer. *Nucl Oncol* 2016; 1-21.
14. Yuka Y, Reiko K, Kunihikol et al. Detection of colorectal cancer using F-18-FLT PET: comparison with F-18-FDG PET. *Nucl Med Commun* 2009; 30(11): 841-5.
15. Helmut RM, Michael H, Uwe H. ^{68}Ga-Labeled Peptides in Tumor Imaging. *J Nucl Med* 2005; 46: 172-8.
16. Chadha VD, Dhawan D. ^{65}Zn kinetics as a biomarker of DMH induced colon carcinogenesis. *Hell J Nucl Med* 2010; 13(3): 257-60.

Department of Error
In the previous issue of Hell J Nucl Med vol. 22 page 77, the headboard of this page should be replaced by: ***Images.***