Biological Effects Associated with Internal and External Contamination of Diagnostic Nuclear Medicine Sources: An In vitro Study

Abstract

Aim: In a Nuclear Medicine department, the risk of external and internal contamination in radiation workers is much higher than in other medical radiation facilities. The risk associated with both types of contaminations should be quantified to estimate the radiation dose received by the personnel. Here, we designed an in vitro model to see the impact of internal and external contamination of F-18 and Technetium-99 m (Tc-99 m) on DNA damages. Methodology: Chinese hamster lung fibroblast V79 was used for all of the experiments. Irradiation was performed internally and externally (scenarios activity is mixed with the cell line [Internal] and activity kept at 1 cm distance from cell line [external]) using two different diagnostic radioactive sources (Tc-99 m and F-18) of known quantity 37 MBq. Total cumulated activity (MBq-min) was calculated up to one half-life of sources for both internal and external setups. An alkaline single gel electrophoresis technique (comet assay) was used for DNA damage analysis. Olive tail moment (OTM) was used to characterize DNA damage. Results: We have not observed any significant difference (P > 0.05) in OTM between internal and external irradiation for cumulated activity presented before one half-life of both diagnostic isopes. However, a significant difference in OTM was noted between internal and external irradiation for cumulated activity presented at one half-life of radioactive sources (P < 0.05). DNA damage with internal exposure was found to be 17.28% higher for F-18 and 23% higher for Tc-99 m than external exposure at one half-life of radioactive sources. Overall, we noted greater DNA damage in F-18 as compared to Tc-99 m. Conclusions: Our in vitro study practically demonstrated that internal contamination is more hazardous than external exposure.

Keywords: 18F-fluorodeoxyglucose, 99 mTcO4-, DNA damage, external contamination, internal contamination

Introduction

Ionizing radiation can displace electrons from atom when they interact with orbits in the atom. Similarly, ionizing radiation also interacts with cells or biological tissue and introduces several damages. The severity of these damages mainly depends on the dose absorbed by the body organs or tissues.[1] The most extensively investigated damage is the DNA double-strand breaks (DSBs).[2] DNA damage can induce apoptosis, genomic instability, and mutations in the cell.

The dose received by a radiation professional mainly depends upon the amount of radiation exposure to ionizing radiation. There are two common types of radiation exposures: external and internal.[3] External exposure mainly takes place when the radiation source is located outside the body. In Nuclear Medicine, the origins of external exposure are patient handling, radiopharmaceutical preparation, dispensing, injection, and surface contamination. Internal exposure occurs when a radioactive material enters the body through different modes, i.e., inhalation, ingestion, absorption, or injection.

The majority of nuclear medicine applications involve unsealed radioactive sources, which are a major source of radiation exposure. While handling unsealed radioactivity, the chances of skin contamination are much higher. Besides, such contaminations can deliver radiation dose both internally and externally. Internal contamination only takes place when the activity gets absorbed through the skin and enters inside the body. An increase in percutaneous absorption may induce more damages to biological tissue or internal organs. External contamination takes place when the component of the radioactive material is deposited on the skin or the body surface.

Address for correspondence:
Dr. Deepanjan Mitra,
Institute of Nuclear Medicine and Molecular Imaging,
AMRI Hospitals, P-4 and 5, C.I.T Scheme-LXXII,
Block-A, Gariahat Road,
Dhakuria, Kolkata - 700 029,
West Bengal, India.
E-mail: deepsjaan@gmail.com
Received: 07-02-2021
Revised: 19-03-2021
Accepted: 29-05-2021
Published: 23-09-2021

Access this article online

Website: www.ijnm.in
DOI: 10.4103/ijnm.ijnm_17_21

How to cite this article: Nautiyal A, Mondal T, Goel A, Dey SK, Mitra D. Biological effects associated with internal and external contamination of diagnostic nuclear medicine sources: An In vitro study. Indian J Nucl Med 2021;36:288-92.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHRRPMedknow_reprints@wolterskluwer.com
A limited number of studies reveal the biological effects associated with the contamination from diagnostic isotopes used in nuclear medicine. In a previous study, it has been demonstrated that F-18 fluorodeoxyglucose (F-18 FDG) can induce DNA damage followed by cellular apoptosis if body cells and tissues are exposed for a longer period.[4] Some published data have also shown radiation DNA damage in an in vivo and in vitro model postnuclear medicine procedures.[3,5-11]

It is well known that in the Nuclear Medicine department, the chances of radioactive spillage and skin contamination are much higher than in other medical radiation modalities. Therefore, the risk of such contamination should be well known and quantified. Here, we have designed an in vitro model to see the impact of internal and external contamination on DNA damages. A single gel electrophoresis technique (SGET) was used for DNA damage analysis. The study was performed using F-18 and Technetium-99 m sodium pertechnetate (Tc-99 m) sources, the workhorse of Nuclear Medicine laboratories.

**Methodology**

**Cell culture**

The cell line used in this study was Chinese hamster lung fibroblast V79 (ATCC, US). (Dulbecco’s modified Eagle’s medium; Gibco, Grand Island, NY) was used to maintain these cell lines as a monolayer. For the supplement, 10% (fetal bovine serum; Gibco), and 100 U penicillin + 100 μg streptomycin/ml (Gibco) were used. Cells were cultured in two different 75 cm² flasks with a humidified atmosphere, 5% CO2 at 37°C. The density at which these cells seeded was 106 cells/flask and subsequently irradiated using F-18 FDG and Tc-99 m sources in cold buffer conditions for three-time points. Irradiation for both sources was performed separately. Besides, cells were irradiated using an internal and external method for three different time points. During all experimental procedures, an untreated control was used.

**Irradiation**

Irradiation was performed using two different diagnostic radioactive sources, Tc-99 m, and F-18.

**External irradiation**

Irradiation was performed in a nonradioactive area of the department under shielding conditions. Two different experiments with two different sources (F-18 FDG and Tc-99 m) of known quantities (37 MBq diluted in 1 ml saline) were used for external irradiation [Figure 1]. Here radioactive sources were placed externally at 1 cm distance from cell line. Cells were irradiated for one physical half-life of sources i.e., 2 h and 6 h (F-18 and Tc-99 m, respectively). During external irradiation of cells with F-18, samples were collected at 10, 60, and 120 min postadministration. Similarly, during irradiation of cells with Tc-99 m, samples were collected at 30, 180 and 360 min postinjection. Cells were irradiated under cold buffer conditions at a distance of 1 cm from the radioactive sources, whereas a thin glass medium (2 mm) was kept between the cell line and radioactive sources to separate each other.

**Internal irradiation**

Internal irradiation was performed with radioactive sources mixed uniformly with the cell lines [Figure 1]. All cells were irradiated separately using 37 MBq of F-18 and Tc-99 m. During the experiment, cells were kept under cold buffer conditions and subsequently collected at 10, 60, and 120 min postadministration of F-18 and 30, 180, and 360 min postadministration of Tc-99 m.

**Cumulated activity estimation**

Total cumulated activity (time-integrated activity) (MBq-min) was calculated for the time as mentioned earlier for both internal and external experiments. Using the known physical half-life of a radioactive source, decayed activity was estimated for each minute until the irradiation of cells (120 min and 360 min for F-18 and Tc-99 m, respectively). Thereafter, cumulated activity at sample collection time points was estimated simply by the integration of activity.

**Single gel electrophoresis technique**

All irradiated cells were processed for DNA damage analysis immediately after the collection at 10, 60, and 120 min postirradiation of F-18 and 30, 180 and 360 min postirradiation of Tc-99 m. An alkaline SGET (comet assay) was used for DNA damage analysis. The same method was used as described by Mondal et al.[12] During both experiments, irradiated cells and negative control (non-irradiated cells) were kept for the study. All slides of SGET were coated with agarose (normal melting point). After the coagulation of the first agarose layer, 100 ul of a mixture of agarose (low melting point) and 80 ul of a PBMC mixture was
coated as a second layer. Electrophoresis was performed for 30 min at 300 mA at 24V. A Carl Zeiss fluorescence microscope (Axioskop 40) was used to observe the DNA damage. All comet presenting cells were analyzed using CASP software (CASP, Wroclaw, Poland). Subsequently, to characterize DNA damage in the form of olive tail moment (OTM), tail length and percentage of DNA in the comet tail were recorded.

**Statistical analysis**

All quantitative parameters are expressed as mean ± standard deviation. In addition, a paired t-test was performed using Origin Pro 2019 (OriginLab Corp., Northampton, MA, USA) to measure the level of significance between internal and external irradiation. Statistically, a significant difference was considered for a \( P = 0.5 \).

**Results**

A SGET was used to estimate DNA damage in cells. Internal and external irradiation was performed using known quantities of radioactive sources. With increasing time, cumulated activity also increased for both sources. In our study, the frequency of appearance of comet cells changed with cumulated activity and both internal and external irradiation [Figure 2].

For the F-18 source, cumulated activity noted at 10, 60, and 120 min was 87.7, 225.7, and 325.6 MBq-min, respectively [Figure 2a]. Similarly, for the Tc-99 m source, cumulated activity noted at 10, 60, and 120 min was 142.3, 589.96, and 961.46 MBq-min, respectively [Figure 2b]. We did not observe any significant difference (\( P > 0.05 \)) in OTM between internal and external irradiation for cumulated activity presented before one half-life of both diagnostic isotopes. However, a significant difference in OTM was noted between internal and external irradiation with cumulated activity presented at one half-life of radioactive sources (\( P < 0.05 \)) [Tables 1 and 2].

DNA damage with internal exposure was found to be 17.28% higher for F-18 and 23% higher for Tc-99 m as compared to external exposure at one half-life of radioactive sources. We have noted higher DNA damage with F-18 as compared to Tc-99 m. At one physical half-life, OTM observed with external irradiation was 1.86 times higher in F-18 than that in Tc-99 m. Similarly, OTM associated with internal irradiation was found to be 1.77 times higher in F-18 than that in Tc-99 m.

**Discussion**

F-18 and Tc-99 m are the most commonly used isotopes in Nuclear Medicine. These isotopes are used in an unshielded liquid form and labeled with various pharmaceuticals for single-photon emission computed tomography and PET applications. Therefore, the chances of contamination are much higher compared to other radiation modalities. Internal

---

**Table 1: Comparison of DNA damage between internal and external irradiation for different cumulated activity of F-18**

| Time (min) | Cumulated activity (MBq-min) | OTM External | OTM Internal | \( P \) |
|-----------|-------------------------------|--------------|--------------|------|
| 10        | 87.7                          | 0.73±0.09    | 0.83±0.13    | 0.06 |
| 60        | 225.7                         | 21.41±3.85   | 28.9±7.89    | 0.3  |
| 120       | 325.6                         | 78.7±14.50   | 92.3±15.93   | 0.01 |

Results displayed are mean±SEM. SEM: Standard error of the mean, OTM: Olive tail moment

**Table 2: Comparison of DNA damage between internal and external irradiation for different cumulated activity of Tc-99m**

| Time (min) | Cumulated activity (MBq-min) | OTM External | OTM Internal | \( P \) |
|-----------|-------------------------------|--------------|--------------|------|
| 30        | 142.3                         | 0.42±0.18    | 0.65±0.11    | 0.3  |
| 180       | 589.96                        | 14.89±3.94   | 18.88±6.40   | 0.12 |
| 360       | 961.46                        | 42.22±5.22   | 51.96±8.22   | 0.04 |

Results displayed are mean±SEM. SEM: Standard error of the mean, OTM: Olive tail moment
and external contamination may induce significant physical effects because ionizing radiation can ionize molecules and atoms and break DNA. Internal exposure is known to have a higher risk compared to external exposure.\[13]\] This risk is mainly because of the higher absorbed dose in organs or tissues. In addition, the greater DNA damage is associated with a higher accumulated absorbed dose in tissues.\[14]\]

In the present study, we developed an in vitro model for external and internal irradiation and subsequently explored the effect of both internal and external contamination of F-18 and Tc-99 m on DSBs.

In nuclear medicine applications, the risk of external contamination is much higher as compared to internal contamination. However, contaminated externally, professionals can become internally contaminated if radioactivity enters into their body, for example, by skin absorption or by placing hands inside the mouth.\[15,16]\] Authors have revealed that internal contamination is more hazardous than external contamination.\[16,17]\]

The severity of effects mainly depends on the type of isotope, its half-life, amount of activity, number of emissions, energy, and total exposure time.\[18]\] Unlike other radiation specialties, nuclear medicine applications possess a high risk of contamination, which is a significant source of exposure.\[19]\] We observed higher DNA damage with internal irradiation compared to external irradiation from Tc-99 m and F-18 sources in the present study. Radiation exposure and absorbed dose from internal contamination can induce greater DNA damage than external contamination.\[20]\] However, in the case of internal contamination, all of these radiations can directly interact with body tissues or organs and eventually deliver more radiation dose. In internal contamination, the energy of other emissions like beta or alpha and auger electrons is also absorbed by tissues, making internal exposure more hazardous than external exposure. Besides, the internal dose to body tissues also depends upon the total exposure time and elimination of radioactive sources from the body by decay and excretion. Studies have shown that Tc-99 m auger electrons can enhance DNA damage.\[21]\] In an in vitro study, induction of DNA damage was noted by the Tc-99 m source in MNBC cells.\[22]\] Similarly, several in vivo and in vitro studies have demonstrated the severity of biological effects with F-18 labeled pharmaceuticals.\[2,3,8,23]\] The risk of internal contamination through the skin and associated radiation exposure and dose can be minimized by adopting rapid decontamination procedures.\[24]\]

In the present study, we have also found that DNA damage from both internal and external irradiation increases with different levels of cumulated activity of diagnostic isotopes. In an individual, if these damages do not repair correctly at any point, they may lead to cell death or eventually become cancerous. Mondal et al. also noted a higher number of DNA lesions with increasing dose and irradiation time of F-18 FDG external exposure.\[24]\] Since the study was performed up to one half-life of isotopes, therefore further effects can be assessed by extrapolation beyond one half-life of Tc-99 m and F-18.

We have noted that F-18 can induce a more significant number of DNA damages than Tc-99 m regardless of lower half-life, both internally and externally. These damages are mainly because F-18 decays by beta plus mode, which produces two annihilated higher energy gamma photons (511 keV).\[23]\] On the other hand, unlike F-18, Tc-99 m emits a low energy gamma-ray (140.5 keV) along with low energy auger and conversion electrons.\[26]\] Besides, beta radiation has relatively higher linear energy transfer than gamma radiation which can amplify biological effects at a greater level.

Our in vitro study is designed to demonstrate the overall effects of internal and external contamination on living cells or tissues of radiation workers working in Nuclear Medicine. However, the real scenario could be different, because of the body’s own repair mechanism to repair the radiation-induced damages, which vary from individual to individual. Therefore, future studies may be performed to see overall repair time and process post internal and external irradiation.

**Conclusions**

Our in vitro study practically demonstrated that internal contamination creates more DNA damages than external contamination. In a scenario, F-18 can induce 1.86 times higher DNA damage than Tc-99 m if external contamination takes place and 1.77 times greater DNA damage than Tc-99 m if internal contamination takes place. DNA damages are directly associated with increasing irradiation time and the amount of dose delivered from cumulated activity. Therefore, careful application and safe handling of nuclear medicine sources are of utmost importance to prevent radioactive contamination and related biological effects.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Feinendegen LE, Pollycove M, Sondhaus CA. Responses to low doses of ionizing radiation in biological systems. Nonlinearity Biol Toxicol Med 2004;2:143-71.
2. Mondal T, Nautiyal A, Agrawal M, Mitra D, Goel A, Kumar Dey S. 18F-FDG-induced DNA damage, chromosomal aberrations, and toxicity in V79 lung fibroblast cells. Mutat Res 2019;847:503165.
3. Misra RS, Johnston CJ, Groves AM, DeDiego ML, St Martin J, Reed C, et al. Examining the effects of external or internal radiation exposure of juvenile mice on late morbidity after
infection with influenza A. Radiat Res 2015;184:3-13.
4. Mondal T, Nautiyal A, Patwari A, Ozukum A, Mitra D, Goel A, et al. DNA double strand breaks, repair and apoptosis following 511 keV γ-rays exposure using 18F positron emitter: An in vitro study. Mutagenesis 2014;29:279-87.
5. Taylor K, Jennifer AL, Douglas RB. Radiation-induced DNA damage and the relative biological effectiveness of 18F-FDG in wild-type mice. Biochem Biophys Res Commun 2014;454:7-11.
6. Nautiyal A, Mondal T, Mukherjee A, Mitra D, Kaushik A, Goel HC, et al. Quantification of DNA damage in patients undergoing non‑contrast and contrast enhanced whole body PET/CT examinations using comet assay and micronucleus assay. Int J Radiat Biol 2019;95:710-9.
8. May MS, Brand M, Wuest W, Anders K, Kuwert T, Prante O, et al. Induction and repair of DNA double-strand breaks in blood lymphocytes of patients undergoing 18F-FDG PET/CT examinations. Eur J Nucl Med Mol Imaging 2012;39:17129.
9. Dantas FJ, de Mattos JC, Moraes MO, Boasquevisques E, Rodrigues MP, Lage CA, et al. DNA damage in peripheral blood nuclear cells assessed by comet assay from individuals submitted to scintigraphic examinations. Cell Mol Biol (Noisy-le-Grand) 2002;48:789-91.
10. Pedraza-López M, Ferro-Flores G, Mendiola-Cruz MT, Morales-Ramírez P. Assessment of radiation-induced DNA damage caused by the incorporation of 99mTc-radiopharmaceuticals in murine lymphocytes using single cell gel electrophoresis. Mutat Res 2000;465:139-44.
11. Kara P, Dağdeviren K, Özsoz M. An electrochemical DNA biosensor for the detection of DNA damage caused by radioactive iodine and technetium. Turk J Chem 2007;31:243-9.
12. Mondal T, Pal S, Dey SK. Quercetin mediated inhibition of hydrogen peroxide-induced genomic DNA damage and toxicity. J Biol Active Nat Prod 2017;7:200-13.
13. Institute of Medicine (US) Committee on Battlefied Radiation Exposure Criteria. Johnson JC, Thaul S, editors. An Evaluation of Radiation Exposure Guidance for Military Operations: Interim Report. Washington, DC, USA: National Academies Press; 1997. p. 2.
14. Desoukya O, Ding N, Zhou G. Targeted and nontargeted effects of ionizing radiation. J Radiat Res Appl Sci 2015;8:247-54.
15. Drozdovitch V, Khrouch V, Minenko V, Konstantinov Y, Khrutchinsky A, Kutsen S, et al. Influence of the external and internal radioactive contamination of the body and the clothes on the results of the thyroidal 131I measurements conducted in Belarus after the Chernobyl accident. Part 1: Estimation of the external and internal radioactive contamination. Radiat Environ Biophys 2019;58:195-214.
16. Arora R, Chawla R, Marwah R, Kumar V, Goel R, Arora P, et al. Medical radiation countermeasures for nuclear and radiological emergencies: Current status and future perspectives. J Pharm Bioalied Sci 2010;2:202-12.
17. Choi TA, Costes SV, Abergel RJ. Understanding the health impacts and risks of exposure to radiation. In: Ahn J, Carson C, Jensen M, Jurakí K, Nagasaki S, Tanaka S, editors. Reflections on the Fukushima Daiichi Nuclear Accident. Cham: Springer, 2015.
18. Reisz JA, Bansal N, Qian J, Zhao W, Furdui CM. Effects of ionizing radiation on biological molecules – mechanisms of damage and emerging methods of detection. Antioxid Redox Signal 2014;21:260-92.
19. Wang HB, Zhang QZ, Zhang Z, Hou CS, Li WL, Yang H, et al. Necessity of internal monitoring for nuclear medicine staff in a large specialized Chinese hospital. Int J Environ Res Public Health 2016;13:418.
20. Melzer P. Radiation dose-rate and DNA damage. Environ Health Perspect 2012;120:A417-8.
21. Belchior A, Di Maria S, Fernandes C, Vaz P, Paulo A, Raposinho P. Radiobiological and dosimetric assessment of DNA-intercalated 99mTc-complexes bearing acridine orange derivatives. EJNMMI Res 2020;10:79.
22. Hengstler JG, Bockisch A, Fuchs J, Grimm W, Görges R, Oesch-Bartlomowicz B, et al. Induction of DNA single-strand breaks by 131I and 99mTc in human mononuclear blood cells in vitro and extrapolation to the in vivo situation. Radiat Res 2000;153:512-20.
23. Schmeiser HH, Muehlbauer KR, Mier W, Baranski AC, Neels O, Dimitrakopoulou-Strauss A, et al. DNA damage in human whole blood caused by radiopharmaceuticals evaluated by the comet assay. Mutagenesis 2019;34:239-44.
24. International Atomic Energy Agency, Assessment and Treatment of External and Internal Radionuclide Contamination, IAEA-TECODC-869. IAEA, Vienna, 1996.
25. Cole EL, Stewart MN, Littich R, Hoareau R, Scott PJ. Radiosyntheses using fluorine-18: The art and science of late stage fluorination. Curr Top Med Chem 2014;14:875-900.
26. Pomplun E, terrissol M, Kümmerle E. Estimation of a radiation weighting factor for 99mTc. Radiat Prot Dosimetry 2006;122:80-1.