

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 personal. 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 isotopes. 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.

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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

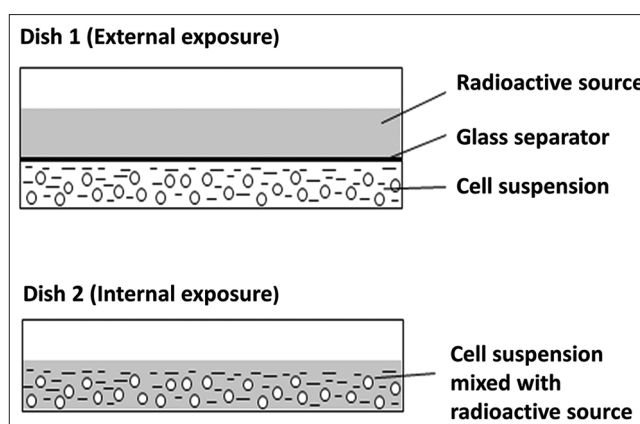


Figure 1: Experimental setups for internal and external irradiation of cell lines with nuclear medicine sources

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 µl of a mixture of agarose (low melting point) and 80 µl 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

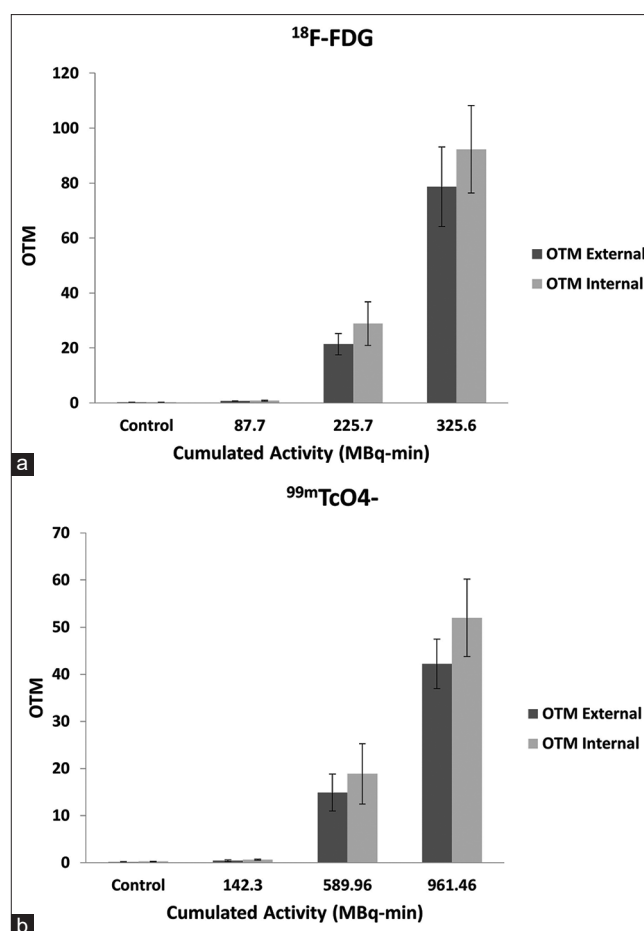


Figure 2: (a) Number of averaged olive tail moment scored from control and different fraction of cumulated activities (up to one half-life of F-18) with external and internal irradiation setup. (b) Number of averaged olive tail moment scored from control and different fraction of cumulated activities (up to one half-life of technetium-99 m) with external and internal irradiation setup

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		<i>P</i>
		External	Internal	
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		<i>P</i>
		External	Internal	
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.^[2,5,8,23] 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.^[4] 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).^[25] 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.

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Conflicts of interest

There are no conflicts of interest.

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