Evaluation of the radiation-sensitizer/protector and/or antioxidant efficiencies using Fricke and PAG dosimeters

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Abstract. In this study, our aim is to assess the potential of Fricke and polyacrylamide gel (PAG) dosimeters to quantitatively evaluate the efficiency of potential radiation sensitizers/protectors and antioxidants. These compounds are of importance in radiotherapy as well as in disease prevention and promotion of health. The basic principle of the Fricke dosimeter is the radiation-induced oxidation of Fe$^{2+}$ to Fe$^{3+}$ in an aerated aqueous 0.4 M $\text{H}_2\text{SO}_4$. The production of ferric ions is most sensitive to the radical species produced in the radiolysis of water. Using this method, we observed that cystamine (one of the best of the known radioprotectors) can prevent oxidation of Fe$^{2+}$ from reactive radiolysis species. However, one obvious disadvantage of the Fricke dosimeter is that it operates under highly acidic conditions (pH 0.46), which may degrade biological compounds. In contrast, the pH of the polyacrylamide gel (PAG) dosimeter is almost neutral, such that degradation of compounds is less probable. A change in $R_2$-dose sensitivity was observed in the presence of radiosensitizers/radioprotectors and antioxidants. The protective effect of Trolox (a well-known antioxidant) and thiourea (a radioprotector) was readily observed using the PAG dosimeter. Incorporation of iodinated radiation sensitizers such as NaI and an iodine contrast agent led to a quantifiable sensitizer enhancement ratio. These studies suggest that the Fricke and the PAG dosimeters have the potential to evaluate the efficiency of radiation sensitizers/protectors and antioxidants.

1. Introduction

Radiation sensitizers and radiation protectors play an important role in clinical radiotherapy [1,2]. It is therefore important to understand the mechanisms of the action of such compounds in order to help better control their biological effects. For example, a chemical compound designed at protecting DNA from radiation damage needs not only to reach its cellular target location but also retain its radioprotecting activity to produce an effective response. Using clonogenic assays, the radiation survival of cells can be measured, but this only provides information on the addition of these two properties. If a compound proves to be less effective than anticipated, is it because it is in fact not a good radioprotector, or is it that it did not reach its target location? This study aims at characterizing compounds at the molecular or chemical level, such that this information can aid in the design and the optimization of compounds that will be therapeutically useful in vivo.

Radiosensitizers are intended to enhance tumor cell killing while having little effect on normal tissues. Enhancement of the radiation damage by radiosensitizers (also called “dose enhancers”)
originates from the direct action of radiation on the sensitizing compounds. This mechanism of radiosensitization can generate a variety of toxic effects: production of secondary electrons and Auger electron cascades [3], X-ray fluorescence [3], high-LET $\alpha$-particles and charged fragments [4] as well as free-radical species [5]. These products can damage critical cellular targets such as DNA, RNA, proteins or lipids, increasing the extent of initial radiation injury and thus improving tumor killing. Inversely chemical radioprotectors act by a decreasing radiosensitivity, especially of normal tissues [6]. This may be associated to the capacity of the radioprotector to scavenge free radicals produced by the ionizing radiation (antioxidant activity).

In this study, we have developed a method, fast and easy to apply, to assess the efficiencies of radiation sensitizers and protectors (antioxidants), based on the use of the Fricke and polyacrylamide gel dosimeters.

1.1. The Fricke dosimeter
The Fricke dosimeter has found extensive use because it gives absolute results with a high accuracy. The basic principle of this chemical dosimeter is the radiation-induced oxidation of Fe$^{2+}$ to Fe$^{3+}$ in an aerated aqueous H$_2$SO$_4$ solution. The production of ferric ions is most sensitive to the radical species produced in the radiolysis of water. The presence of any other compounds during irradiation may affect the availability of those free radicals and will therefore change the radiation chemical yield of Fe$^{3+}$ [$G(Fe^{3+})$]. Normally, under low-linear-energy-transfer (LET) irradiation conditions, $G(Fe^{3+})$ is equal to 15.6 $\pm$ 0.3 molecules/100 eV. In the presence of a radioprotector (or an antioxidant), $G(Fe^{3+})$ is expected to be lower than 15.6, while in the case of a radiosensitizer, it is expected to be higher than 15.6. Using this method, Jayson and Wilbraham [7] have studied the protective action of cystamine, a well-known biological radiation-protective compound. Their results showed that cystamine can prevent oxidation of Fe$^{2+}$, as observed by a lower yield of Fe$^{3+}$. In this case, cystamine and Fe$^{2+}$ competed in the reaction with oxidizing radicals. The Fricke dosimeter was used more recently by Herold et al. [8] who measured a dose enhancement from gold microspheres in solutions irradiated with 200 kVp X-rays and $^{137}$Cs $\gamma$-rays. Together, these studies suggest that the Fricke dosimeter could be a useful tool to evaluate the radiation protective (antioxidant) and radiosensitizing efficiency of water-soluble compounds.

1.2. The polyacrylamide gel (PAG) dosimeter
The PAG dosimeter is a three-dimensional radiation dosimeter that is composed of monomers such as acrylamide (AA) and N,N’-methylene-bis-acrylamide (BIS) dissolved in an aqueous gelatin matrix [9]. The principle of the dosimeter is based on the polymerization of the monomers initiated by radical species produced by the radiolysis of water [10]. In the evaluation of polymer gel dosimeters by magnetic resonance imaging (MRI), the spin-spin (or transverse) relaxation time ($T_2$) of protons in the gel following irradiation can be determined in two or three dimensions. The quasi-linear relationship between the inverse of $T_2$ ($R_2$) and the absorbed dose, at low doses, is called the “$R_2$-dose sensitivity” [11]. More radical species are formed when a radiosensitizer (or “dose enhancer”) is added to the gel dosimeter and this should increase the extent of polymerization for a given dose. Under these conditions, the $R_2$-dose sensitivity will be increased. In a recent study, Boudou et al. [12] have used this method to measure the dose enhancement in polymer gels doped with iodine (a high-Z element). Unlike radiosensitizers, radioprotectors and antioxidants can scavenge radiation-induced water free radicals, such that their presence is expected to lead to a decrease of the $R_2$-dose sensitivity.

2. Experimental procedure

2.1. Fricke dosimeter
The chemicals used in the Fricke dosimeter solution are ferrous ammonium sulfate (as the hexahydrated salt) (99.99 %, Sigma Aldrich), sulfuric acid (98%, Sigma Aldrich), and double-distilled water. The solutions were prepared from 0.342 g of Fe(NH$_4$)$_2$(SO$_4$)$_2$.6H$_2$O dissolved in 0.4 M H$_2$SO$_4$. 

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The concentration of ferrous ions in the solution was $1 \text{ mM}$ and the density of the solution was $1.024 \text{ g/cm}^3$. Special attention was given to cleaning glassware according to the standard procedures used in radiation chemistry.

The samples were irradiated up to $80 \text{ Gy}$ at $25^\circ\text{C}$ by $^{60}\text{Co} \gamma$-rays ($1.25 \text{ MeV}$) in a Gammacell 220 (Atomic Energy of Canada Limited). The dose rate was $3.44 \text{ Gy/min}$.

The concentration of ferric ions was calculated from the increase in optical density (absorbance) measured by UV spectrophotometry at $304 \text{ nm}$ using a value of $2205 \text{ M}^{-1}\text{cm}^{-1}$ for the molar extinction coefficient of $\text{Fe}^{3+}$. $G(\text{Fe}^{3+})$ was obtained from the gradient of the linear curve between the $\text{Fe}^{3+}$ concentration and the absorbed dose.

2.2. PAG dosimeter

The dosimeter gels were prepared with acrylamide (AA) and N,N’-methylene-bis-acrylamide (BIS, 99+%, electrophoresis grade, Aldrich), gelatin (~300 bloom, Aldrich), and water (de-ionized). Gelatin was added to water at room temperature and left to soak for 10 min and subsequently heated and maintained at $45^\circ\text{C}$. The monomers (AA and BIS) were successively added and magnetically stirred for typically 15 min until complete dissolution. The gels were prepared under a controlled $\text{N}_2$ atmosphere inside a glove box. The solution was poured into glass vials having a Teflon-lined screw-cap [10,11].

The samples were irradiated up to $50 \text{ Gy}$ at $25^\circ\text{C}$ in the $^{60}\text{Co}$ Gammacell 220 (see above) or with a 150 kVp X-ray therapy system (Therapax HF150T) whose dose rate was $0.86 \text{ Gy/min}$.

The $T_2$ relaxation times of water protons in the gel samples were determined using a head coil in a Siemens Sonata 1.5 T MRI scanner. The sample vials were imaged with a single slice in the coronal (horizontal) plane at $21^\circ\text{C}$ using a multi-echo spin-echo pulse sequence. The $R_2$ ($R_2 = 1/T_2$)-dose sensitivity was obtained for gels containing various concentrations of selected compounds.

3. Results and discussion

3.1. Fricke dosimeter: Effect of cystamine (a radioprotector/antioxidant)

Figure 1 shows a marked decrease of $G(\text{Fe}^{3+})$ upon addition of cystamine in the presence or in the absence of oxygen in the Fricke dosimeter. Our results clearly confirm the earlier data of Jayson and Wilbraham [7] showing the radiation protective property of this compound.

![Figure 1](image_url)

**Figure 1.** Plot of $G(\text{Fe}^{3+})$ as a function of the concentration of added cystamine in the presence and in the absence of oxygen.

3.2. PAG dosimeter: Effect of Trolox (a radioprotector/antioxidant)

Trolox is a water-soluble tocopherol (vitamin E) analogue, well-known for its antioxidant properties. The data obtained with the PAG dosimeter irradiated with $^{60}\text{Co} \gamma$-rays in the presence of various
concentrations of Trolox show a clear decrease in the $R_2$-dose sensitivity, which varies from 0.31 to 0.20 (± 0.01) s$^{-1}$ Gy$^{-1}$ in the dose range ~1-5 Gy (see Fig. 2). Similar results were observed with thiourea, another radiation-protective compound (results not shown). These results provide convincing evidence of the reliability of our method of protection evaluation.

![Figure 2](image)

Figure 2. Plot of $R_2$ against absorbed dose for two different concentrations of Trolox.

3.3. PAG dosimeter: Effect of NaI and an iodinated contrast agent (ICA) (two radiosensitizers)

Several studies have reported the radiosensitization properties of iodine and some iodinated compounds for clinical use, such as iododeoxyuridine (IUdR) [13]. We first monitored the effect of iodine by adding sodium iodide (NaI) in various concentrations to the dosimeter gel and irradiating the system with X-rays from the Therapax HF150T machine. The average energy of photons was ~40 keV. Typically, at an absorbed dose of 5 Gy, the results shown in Fig. 3a indicate that the $R_2$-dose sensitivity decreases (taking the control line in the absence of NaI as a reference) at the lowest NaI concentration considered (0.01 M) and then increases at our highest concentrations (0.05 and 0.1 M).

This peculiar behavior can be explained as follows. At low enough concentrations, NaI exhibits protective properties. Indeed, in neutral water, NaI is dissociated into Na$^+$ and I$^-$ such that I$^-$ can rapidly scavenge (via electron-transfer-type reactions) the radiolytically formed OH radicals [14] thus preventing the initiation of the polymerization reaction. However, as the NaI concentration increases, the direct action of the radiation on iodine becomes progressively dominant and radiosensitizing effects can be observed. This interpretation was confirmed by using iothalamate meglumine.

![Figure 3](image)

Figure 3. Plot of relative $R_2$ against absorbed dose for three different concentrations of (a) NaI and (b) iodinated contrast agent (ICA).
(Conray®° 30), an ICA, where the iodine atom is covalently bound to the molecule and is therefore not present as I in the gel dosimeter. Figure 3b shows a clear increase of the $R_2$-dose sensitivity from 0.23 s$^{-1}$ Gy$^{-1}$ in the absence of ICA to 0.42 s$^{-1}$ Gy$^{-1}$ at an ICA concentration of 0.094 M. At a given radiation dose, this sensitizing property of ICA increases as the drug concentration increases, as expected.

4. Conclusion

We conclude from these experiments that the Fricke and the PAG dosimeters can be used to evaluate quantitatively the efficiency of radiation-sensitizers/protectors and antioxidants. Work is currently in progress to confirm the present results in biochemical and biological systems so that the reliability of our method can be firmly established.

References

[1] P. Wardman, Chemical radiosensitizers for use in radiotherapy. Clin. Oncol. 19, 397-417 (2007).
[2] C. K. K. Nair, D. K. Parida and T. Nomura, Radioprotectors in radiotherapy. J. Radiat. Res. 42, 21-37 (2001).
[3] H. H. Ertl, L. E. Feinendegen and H. J. Heiniger, Iodine-125, a tracer in cell biology: Physical properties and biological aspects. Phys. Med. Biol. 15, 447-456 (1970).
[4] R. F. Barth, J. A. Coderre, M. G. H. Vicente and T. E. Blue, Boron neutron capture therapy of cancer: Current status and future prospects. Clin. Cancer Res. 11, 3987-4002 (2005).
[5] P. Wardman, The mechanism of radiosensitization by electron-affinic compounds. Radiat. Phys. Chem. 30, 423-432 (1987).
[6] C. F. Dunne-Daly, Principles of radiotherapy and radiobiology. Semin. Oncol. Nurs. 15, 250-259 (1999).
[7] G. G. Jayson and A. C. Wilbraham, The utilisation of the Fricke dosimeter for evaluating the biological radiation-protective potential of water-soluble organic compounds. Chem. Commun. 641-642 (1968).
[8] D. M. Herold, I. J. Das, C. C. Stobbe, R. V. Iyer and J. D. Chapman, Gold microspheres: A selective technique for producing biologically effective dose enhancement. Int. J. Radiat. Biol. 76, 1357-1364 (2000).
[9] M. J. Maryanski, J. C. Gore, R. P. Kennan and R. J. Schulz, NMR relaxation enhancement in gels polymerized and cross-linked by ionizing radiation: A new approach to 3D dosimetry by MRI. Magn. Reson. Imaging 11, 253-258 (1993).
[10] M. Lepage, A. K. Whittaker, L. Rintoul, S. Å. J. Bäck and C. Baldock, The relationship between radiation-induced chemical processes and transverse relaxation times in polymer gel dosimeters. Phys. Med. Biol. 46, 1061-1074 (2001).
[11] M. Lepage, P. M. Jayasakera, S. Å. J. Bäck and C. Baldock, Dose resolution optimization of polymer gel dosimeters using different monomers. Phys. Med. Biol. 46, 2665-2680 (2001).
[12] C. Boudou, I. Trorèze, J. Rousseau, L. Lamalle, J. F. Adam, F. Estève and H. Elleaume, Polymer gel dosimetry for synchrotron stereotactic radiotherapy and iodine dose-enhancement measurements. Phys. Med. Biol. 52, 4881-4892 (2007).
[13] R. Nath, P. Bongiorni and S. Rockwell, Iododeoxyuridine radiosensitization by low- and high-energy photons for brachytherapy dose rates. Radiat. Res. 124, 249-258 (1990).
[14] M. Quintiliani, Physico-chemical basis of radiosensitization by iodine compounds. Radiat. Phys. Chem. 30, 409-422 (1987).