In Vitro Effects of Gamma Radiation from ^{60}Co and ^{137}Cs on Plasmid DNA

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Abstract The effects of gamma radiation from ^{60}Co and ^{137}Cs on DNA in aqueous solution are studied experimentally. Using an improved plasmid purification protocol and improved electrophoretic gel analysis techniques provided results with relatively small uncertainties. The results are compared with both theoretical and experimental results. In particular, the results obtained here are discussed in the light of recent discussion on supposed differences of the effects induced by gamma radiation from ^{60}Co and ^{137}Cs . We find that the effects of both types of gamma radiation are similar.

Keywords Double-strand breaks · Single-strand breaks · Scavenger-derived radicals · Ionizing radiation · pBSKS + DNA · Gamma effects

Irradiation of purified DNA molecules has been extensively used during the last decades for studying the interaction of ionizing radiation with DNA [1–13]. The results obtained in such *in vitro* experiments are, in many aspects, in qualitative agreement with those from irradiation of cells and tissues. For instance, the increase of cell apoptosis as a function of the delivered dose resembles, qualitatively, the increase of SSB (single strand break) and

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DSB (double strand break) lesions in the DNA molecule obtained in the *in vitro* irradiations [14].

Another correlation is found when comparing the effects of radiations with different linear energy transferred (LET) on DNA *in vitro* and *in vivo*. The *in vitro* experiments show that high-LET radiation is more effective in inducing DSB damages on the DNA than low-LET radiation. Accordingly, upon irradiation of living cells, it is observed that high-LET radiation is more effective in inducing apoptosis than low-LET. These results are usually attributed to the ionizing structure along the radiation path. In fact, high-LET radiation produces a number of free radicals, which is considerably greater than that produced by low-LET radiation on the same path length, and higher densities of free-radicals are more effective in producing more severe damage on DNA molecules, such as DSB [15–23].

The different effectiveness of low and high-LET radiations shows up on dosimetric studies on the relative biological effect (RBE) of the radiation in the observation that for low-LET radiation (below 3.5 keV/ μm) the RBE is equal to one, meaning that they all have the same effectiveness in damaging the cell, while for high-LET radiations (above 175 keV/ μm), this factor may reach values around 20 [24].

Recently, however, a work comparing the effects of gamma rays from ^{60}Co and from ^{137}Cs on plasmid DNA upon *in vitro* irradiation brought some doubts about the relationship between RBE and the induction of DSB lesions in the DNA molecule [13]. According to these authors, the yield for SSB and DSB induced by gamma rays from ^{60}Co differs from the corresponding yield induced by gamma rays from ^{137}Cs for equivalent delivered doses. These results are not in agreement with the equivalence of RBE (equal to 1 for both low-LET gammas) resulting from dosimetric considerations.

This is an important observation not only because of its practical importance, but also because it results from one of the first experiments which compare damages induced by different sources of gamma radiation under the same experimental conditions.

The unexpected results obtained by Leloup *et al.* [13] show that the yields for SSB and DSB after irradiation with ^{137}Cs are higher than those obtained after irradiation with ^{60}Co . The authors suggest that this discrepancy can be due to differences in the spectra of the secondary electrons generated by each gamma.

In the present work, we report results obtained for generation of SSB and DSB on double-stranded supercoiled purified plasmid DNA (pBSKS+) upon irradiation with

Fig. 1 Fractions of plasmid in supercoiled (SC) and linear (Lin) forms measured as a function of gamma irradiation from ^{60}Co

Fig. 2 Fractions of plasmid in supercoiled (SC) and linear (Lin) forms measured as a function of gamma irradiation from ^{137}Cs

gammas from ^{60}Co and ^{137}Cs . As in Leloup *et al.* [13], all other experimental conditions were kept the same for both types of irradiation.

Furthermore, special care was taken in reducing the experimental uncertainties which result mainly from the DNA purification protocol and electrophoretic gel analysis. The improved plasmid purification protocol we used is described in Gouveia [25]. DNA electrophoresis gel analysis was performed by using the GelAnalis software, which is fully described in Milian [26]. The electrophoresis procedure will be described in detail in a forthcoming paper.

Here, we present the main results obtained with ^{60}Co and with ^{137}Cs and compare them to test the conclusions from Leloup *et al.* [13]. Irradiation with either ^{60}Co or ^{137}Cs was performed on aqueous solutions of DNA containing 2, 20, 200 mM glycerol, which was employed in view of its free radical scavenger capacity. In Fig. 1, the fractions of plasmid in supercoiled (SC) and linear (Lin) forms measured after irradiation by gammas from ^{60}Co are shown. The fractions for different scavenging capacities were fitted to exponential and linear equations, respectively. It is clear that glycerol reduced the DNA damage for the

Fig. 3 Yields $G(\text{SSB})$ obtained from the gamma irradiation from ^{60}Co and from ^{137}Cs

Fig. 4 Yields $G(\text{DSB})$ obtained from the gamma irradiation from ^{60}Co and from ^{137}Cs

same delivered dose. In fact, the higher the glycerol concentration, the more difficult it is to break the plasmid, resulting in a larger fraction of SC.

In Fig. 2, the results for irradiations with ^{137}Cs are presented. The same qualitative observations made in the case of ^{60}Co can be made here.

Using the theoretical model proposed by Cowan [27], it is possible to calculate the yields for SSB and DSB, respectively. The resulting yields $G(\text{SSB})$ and $G(\text{DSB})$, obtained from both gamma irradiation from ^{60}Co and from ^{137}Cs comm. are shown in Figs. 3 and 4, respectively. The corresponding data available in the literature are also plotted for comparison. For both types of irradiation, the results obtained in this work are in good agreement with the literature.

Fig. 5 Results for RBE obtained for gamma irradiation from ^{137}Cs in this work, and compared with the values obtained in Leloup *et al.* [13]

The RBE is a well-known dosimetric quantity, and it is usually related to the effects of radiation on living cells. A RBE factor relating the damage induced by radiation of the DNA may be defined as [23],

$$\text{RBE}_{\text{rad.}} = \frac{G(\text{DSB})_{\text{rad.}}}{G(\text{DSB})_{\text{ref.}}},$$

where $G(\text{DSB})_{\text{rad}}$ is the yield for DSB induced by the radiation under consideration, and $G(\text{DSB})_{\text{ref}}$ is the yield for DSB induced by a reference radiation, usually X-rays. In this work, the gamma from ^{60}Co has been chosen as reference radiation, as it has the lowest LET. Figure 5 presents the results for RBE obtained for gamma irradiation from ^{137}Cs , in comparison with the values obtained in Leloup *et al.* [13]. It is possible to observe that the RBE_{Cs} obtained in our case is quite close to one, and despite the small uncertainties, one cannot conclude that it is different from that for ^{60}Co .

These results are in good agreement with those expected from the dosimetric data, where $\text{RBE}_{\text{Cs}} = \text{RBE}_{\text{Co}} = 1$. Also, they are in agreement with the theoretical expectations, for which the effects of gammas from ^{60}Co and ^{137}Cs on DNA *in vitro* should be similar [24].

Considering now the results from Leloup *et al.* [13], one observes that the RBE_{Cs} may differ from RBE_{Co} by a factor ~ 2 , as it is the case in the irradiation of DNA at 2 mM of glycerol, but taking into account the large uncertainties in their data, one observes that the present results are in agreement, for both glycerol concentrations, with the results obtained by those authors. Their conclusion, however, is not supported by the results obtained in this work, as the RBE for gamma irradiation from ^{60}Co and from ^{137}Cs obtained here are equivalent. Such equivalence between RBE_{Co} and RBE_{Cs} obtained here is in agreement with the observation in dosimetric studies.

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