NanOx PREDICTIONS OF CELL SURVIVAL PROBABILITIES FOR THREE CELL LINES*

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NanOx, a new biophysical model developed to predict cell survival probability in the context of hadrontherapy, will be presented in the following. The model takes into account the fully stochastic nature of ionizing radiation by considering dose fluctuations both at nanometric and micrometric scales, and introduces the concept of chemical specific energy. The latter represents the induction of cell death by "non-local" events as the accumulation of cellular oxidative stress or sub-lethal lesions induced by reactive chemical species. Such "non-local" events are complementary to "local" events, which take place at a very localized scale and are considered as lethal since they can singly cause cell death. NanOx predictions for V79, CHO-K1 and HSG cell lines irradiated by carbon ions are in a good agreement with the experimental data. The model is able to describe the effectiveness of ions, including the overkill effect at high LET values via the fit of a limited set of parameters. Moreover, the typical shoulder in cell survival curves is reproduced owing to the introduction of the chemical specific energy which varies with LET.

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1. Introduction

Hadrontherapy is becoming an increasingly attractive modality for cancer treatment due to the favourable depth-dose profile of ions, which allows to reach high tumour conformality while sparing healthy tissues. Moreover, the densely-concentrated energy deposition in the Bragg peak region results in a higher relative biological effectiveness (RBE) of ions with respect to

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photons. RBE_S is determined as the ratio between D_{ref} and D corresponding respectively to the doses needed to achieve a given level of survival S with a reference radiation and the radiation under consideration:

$$\mathrm{RBE}_S = \left(\frac{D_{\mathrm{ref}}}{D}\right)_S . \tag{1.1}$$

Despite its simple definition, RBE depends on multiple parameters related both to the irradiation beam and the cell properties; its determination, which should be realized prior to the treatment, is therefore extremely complicated. To date, clinical facilities all over the world have employed three different methodologies to estimate RBE: an empirical procedure developed at the National Institute of Radiological Sciences in Japan [1, 2], the Local Effect Model (LEM) [3, 4], based on the analysis of the particle track structure at nanometric scale, and the Modified Microdosimetric Kinetic Model (MMKM) [5–7], which attributes the radio-induced DNA damages to the interaction of sub-lethal lesions caused by the energy depositions in domains of micrometric size within the cell nucleus. These approaches, which led to the success of hadrontherapy with carbon ions, present, however, several shortcomings [8–10]. On the one hand, it has been underlined that the determination of the biological effects of ionizing radiation should be derived from the details of the particle track structure on a nanometric scale [9]; on the other, even at such a scale, the stochastic nature of radiation should be taken into account, since dose fluctuations are relevant and should not be drowned in observables expressing average quantities [11]. These considerations motivate the development of alternative frameworks, more consistent with the physico-chemical properties of radiation, to improve the theoretical predictions. We propose herein the construction of a new formalism, NanOx, that addresses some of the flaws in the models currently implemented in the treatment planning systems, and exhibits many innovative features.

2. Theoretical framework

We will introduce only the main principles of the new formalism, since a more complete and rigorous outline can be found in [12]. In order to integrate the stochasticity that ensues from the exposition of biological matter to radiation, NanOx predicts the cell survival probability in terms of the average over all configurations of radiation impacts and irradiated cells. Ion track details and photon interactions are taken into account down to nanometric scale via the spatial distribution of the energy-transfer points and of the physico-chemical events associated to K radiation impacts (such a configuration will be represented by the index c_K in the following). Cells spatial distribution and geometry are as well integrated in the model; NanOx, however, does not give an explicit description of the possible communication

between them (*i.e.* bystander effect), meaning that it actually considers a representative cell in an average cycle stage. As a result, the average cell survival probability is computed by taking into account all the configurations with K radiation impacts, for all the possible values of K that may be achieved with the delivered physical dose D:

$$\overline{S(D)} = \sum_{K=0}^{K=\infty} P(K,D) \cdot \langle {}^{c_K}S \rangle_{c_K} \,, \tag{2.1}$$

where $P(K,D)$ is the probability to have K impacts with a dose D and $\langle {}^{c_K}S \rangle_{c_K}$ is the mean survival probability over all the configurations c_K.

The new framework attributes the process of cell death induction to the separate contributions of two types of biological events taking place at different spatial scales. The probability of cell survival, therefore, can be factorized in a component due to the action of *local lethal events* (${}^{c_K}S_{\mathrm{L}}$), and one due to *non-local events* (${}^{c_K}S_{\mathrm{NL}}$):

$${}^{c_K}S = {}^{c_K}S_{\mathrm{L}} \times {}^{c_K}S_{\mathrm{NL}} \,. \tag{2.2}$$

The two terms appearing in Eq. (2.2) are assumed to be independent and defined in two sensitive volumes that are *a priori* different. The local lethal events are caused by physico-chemical processes in a very localized volume (< 100 nm), inside which the probability that two or more particle tracks deposit a significant specific energy can be neglected at clinical doses [11]. These events represent complex DNA lesions (*e.g.* unrepaired/misrepaired DNA double-strand breaks), and can, alone, lead to cell death. The non-local events are harmful but cannot induce cell death by their own. They represent the accumulation and the interaction, at the microscopic cellular scale, of sublethal damages (*e.g.* DNA single-strand breaks), lesions in different cellular structures (*e.g.* mitochondria, nuclear and cellular membranes) and oxidative stress.

2.1. Cell survival to local lethal events

The modelling at nanometric scale relies on the presence of N local targets distributed uniformly in the sensitive volume. NanOx postulates that a local lethal event is represented by the inactivation of one of such targets, and can be expressed in terms of a function of an observable that characterizes the radiation quality in said local target; in the first version of the model, we designated the specific energy z and estimated it by simulation using the LQD (LiQuiD water radiolysis) Monte Carlo code [13]. Since the local targets responses are independent, the probability of cell survival to

local lethal events for a given configuration of local targets (c_N) and radiation impacts (c_K) is equal to the probability that no local target is inactivated:

$$ {}^{c_N,c_K}S_{\mathrm{L}} = \prod_{k=1}^{K} {}^{c_N,c_k}S_{\mathrm{L}} = \prod_{k=1}^{K}\prod_{i=1}^{N} \left(1 - f\left({}^{c_i,c_k}z\right)\right) . \tag{2.3} $$

f is the inactivation function, ${}^{c_i,c_k}z$ represents the specific energy in the local target i with configuration c_i (*i.e.* position and orientation) after one radiation impact with configuration c_k. The introduction of an effective lethal function F, defined by $F(x) = -N\ln(1-f(x))$, allows to reformulate Eq. (2.3) into:

$$ {}^{c_K}S_{\mathrm{L}} = \prod_{k=1}^{K} \exp\left(-\left\langle F\left({}^{c_i,c_k}z\right)\right\rangle_{c_i}\right) . \tag{2.4} $$

2.2. Cell survival to non-local events

In the first version of NanOx , we decided to represent non-local events by *global* events, which account for the production of chemical reactive species in the associated sensitive volume. Indeed, it has been demonstrated that the latter induce a significant part of DNA sublethal damages [14, 15] and are directly involved in cellular oxidative stress. Several new concepts have been introduced to characterize properly the radiation at such a global scale: firstly, the relative chemical effectiveness, RCE, which represents the ratio of the specific energies deposited by the reference radiation and a given ion causing the same level of oxydatif stress; secondly, the chemical specific energy after a configuration of radiation impacts c_K, which is defined as:

$$ {}^{c_K}\tilde{Z} = \sum_{k=1}^{K} {}^{c_k}\mathrm{RCE} \times {}^{c_k}z\,. \tag{2.5} $$

Both observables, assessed via the LQD, PHYCHEML and CHEM Monte Carlo codes [13, 16, 17], depend on the time T_{RCE} that separates the irradiation and the measurement of the yield of the reactive chemical species, which interact and recombine continuously with each other. The modelling of cell survival probability to global events is realized owing to a linear-quadratic (LQ) expression in terms of the chemical specific energy:

$$ {}^{c_K}S_G\left({}^{c_K}\tilde{Z}\right) = C_{\mathrm{norm}} \times \exp\left(-\alpha_{\mathrm{G}} \times {}^{c_K}\tilde{Z} - \beta_{\mathrm{G}} \times {}^{c_K}\tilde{Z}^2\right) , \tag{2.6} $$

where C_{norm} is a normalization factor ensuring that the average cell survival over all irradiation configurations leads to the experimental probability of cell survival to a reference radiation characterized by the LQ coefficients α_{r} and β_{r}. We chose low-LET photons, emitted from a ^{60}Co source, as reference radiation.

3. Model parameters

To predict the cell survival probability, NanOx requires the introduction of various parameters; some of them need to be adjusted to experimental data, others can be fixed according to some considerations without alterating the result in a significant way. As previously mentioned, the modelling of local lethal events is based on the effective lethal function F. It is out of the scope of this paper to prove that an error function, built via a data-driven procedure, is a good candidate to represent F:

$$F(z) = \frac{h}{2}\left[1 + \left(\frac{z - z_0}{\sigma}\right)\right] . \tag{3.1}$$

The three parameters, z_0 (the threshold), σ (the width of the increase), h (the height of the response) are obtained via the fit of measured α values of a specific cell line irradiated by photons and at least two monoenergetic ion beams characterized by intermediate and high LET values. The modelling of global events (Eq. (2.6)) needs instead as input the experimental β coefficient issued from the LQ fit of cell survival to reference radiation, β_{r}. Indeed, it can be demonstrated that:

$$\beta_{\mathrm{G}} = \frac{\beta_{\mathrm{r}}}{\eta^2} , \tag{3.2}$$

where the factor $\eta = \frac{\langle Z \rangle}{D} \simeq 80\%$ is introduced since the LQD Monte Carlo code discards the deposited energy that simply causes the heating of the medium when estimating the specific energy. Finally, another experimental evidence is required to fix the size of the sensitive volumes associated to the local lethal and global events; in this first version of the model, both are assumed to correspond to the average dimension of the cell nuclei.

Minor parameters, which can be considered almost independent of the cell line, can be fixed: α_{G} defined in Eq. (2.6) is set as 0 to allow for a separate adjustment of local and global events; T_{RCE} is set to 10^{-11} s to roughly characterize the production of primary chemical reactive species [18]; the local targets are defined as cylinders with radius and length equal to 10 nm to match approximately the extension of a DNA DSB [11, 19, 20] and take the diffusion of reactive species also into account [19, 21].

4. Model predictions for different cell lines

To test the potential of the new biophysical model, we compared its predictions to the experimental dose-response curves obtained by irradiating with monoenergetic ion beams three cell lines of different radiosensitivity. We chose human tumour salivary glands cells (HSG) and, due to the

large amount of data available in literature, Chinese hamster lung fibroblasts (V79) and ovary (CHO-K1) cells. For each cell line, we optimized the effective lethal function, obtaining different values for the parameters. The sensitive volume was set as a cylinder of 1 μm lenght and of 7 μm (HSG), 4.9 μm (V79) and 5.9 μm (CHO-K1) radius. Among several sets of LQ coefficients describing the reference radiation issued from different publications, we chose the ones closer to the average values. In the case of CHO-K1 cells, however, it was not possible to identify an "average" experimental $(\alpha_{\mathrm{r}}, \beta_{\mathrm{r}})$ pair, thus we applied a correction factor to the β_{r} coefficient in order to better reproduce the β(LET) distribution for ions. Figure 1 offers a coincise report of the obtained results, the surviving fractions of the three cell lines irradiated by two carbon ion beams of different energies; an overall agreement between NanOx predictions and the measured values is observed for a wide LET range, supporting the robustness of the model.

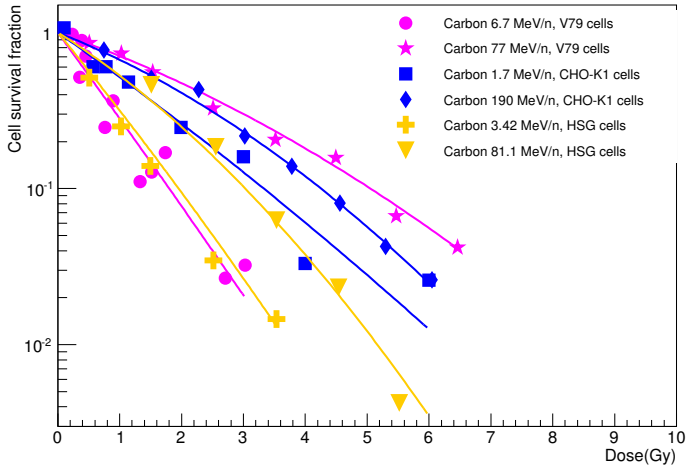

Fig. 1. (Colour on-line) Comparison of cell survival curves determined experimentally (full markers) and using the model (solid lines) for V79 cells (magenta full dot and star), CHO-K1 cells (blue diamond and square), HSG cells (orange triangle and cross) irradiated with carbon ions of low and high LET [22–25].

5. Conclusion

This paper presents a succinct description of a new multiscale biophysical model, NanOx, and the promising results obtained predicting cell survival for three cell lines irradiated by carbon ion beams. Despite a rigorous mathematical approach, its implementation remains simple and compatible with the constraints of clinical application. The model relies in fact on the fit of only 5 parameters, and its pragmatic architecture facilitates further improvements and optimizations.

REFERENCES

[1] Y. Hirao *et al.*, *Nucl. Phys. A* **538**, 541 (1992).
[2] T. Kanai *et al.*, *Radiat. Res.* **147**, 78 (1997).
[3] M. Scholz *et al.*, *Radiat. Environ. Biophys.* **36**, 59 (1997).
[4] M. Kramer, M. Scholz, *Phys. Med. Biol.* **45**, 3319 (2000).
[5] R.B. Hawkins, *Radiat. Res.* **140**, 366 (1994).
[6] R. Hawkins, *Int. J. Radiat. Biol.* **69**, 739 (1996).
[7] Y. Kase *et al.*, *Radiat. Res.* **166**, 629 (2006).
[8] D. Schardt, T. Elsässer, D. Schulz-Ertner, *Rev. Mod. Phys.* **82**, 383 (2010).
[9] M. Beuve, *Radiat. Res.* **172**, 394 (2009).
[10] G. Russo *et al.*, *Radiat. Prot. Dosim.* **143**, 497 (2011).
[11] M. Beuve *et al.*, *Nucl. Instrum. Methods Phys. Res. B* **267**, 983 (2009).
[12] M. Cunha, C. Monini, E. Testa, M. Beuve, *Phys. Med. Biol.* **62**, 1248 (2017).
[13] B. Gervais *et al.*, *Chem. Phys. Lett.* **410**, 330 (2005).
[14] J.-L. Ravanat, T. Douki, J. Cadet, *J. Photoch. Photobio. B* **63**, 88 (2001).
[15] C. von Sonntag, *Free-Radical-Induced DNA Damage and Its Repair*, Springer, Berlin–Heidelberg 2006.
[16] B. Gervais *et al.*, *Radiat. Phys. Chem.* **75**, 493 (2006).
[17] A. Colliaux, B. Gervais, C. Rodriguez-Lafrasse, M. Beuve, *Nucl. Instrum. Methods Phys. Res. B* **365**, 596 (2015).
[18] M.S. Kreipl, W. Friedland, H.G. Paretzke, *Radiat. Environ. Biophys.* **48**, 349 (2009).
[19] T. Elsässer, M. Scholz, *Radiat. Res.* **167**, 319 (2007).
[20] A. Schipler, G. Iliakis, *Nucl. Acids Res.* **41**, 7589 (2013).
[21] H. Nikjoo, P. O'Neill, D.T. Goodhead, M. Terrissol, *Int. J. Radiat. Biol.* **71**, 467 (1997).
[22] M. Belli *et al.*, *J. Radiat. Res.* **49**, 597 (2008).
[23] W. Weyrather *et al.*, *Int. J. Radiat. Biol.* **75**, 1357 (1999).
[24] J. Czub *et al.*, *Int. J. Radiat. Biol.* **84**, 821 (2008).
[25] Y. Furusawa *et al.*, *Radiat. Res.* **154**, 485 (2000).