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Evaluating normal tissue damage after hadrontherapy by chromosome aberration prediction

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Summary. — In cancer hadrontherapy, the Relative Biological Effectiveness (RBE) is calculated by considering cell survival as the endpoint of interest. Although this is a good estimator of the beam effectiveness in eliminating the tumour cells, late damage in healthy tissues, including secondary tumours, is better correlated with chromosomal aberrations. In particular, dicentrics in blood lymphocytes are widely considered as a good biomarker of normal tissue damage, as the blood circulates in all tissues and is inevitably exposed to radiation during the treatment. In this study, lymphocyte dicentrics were used as an *ad hoc* endpoint to evaluate the RBE for (late) normal tissue damage by means of BIANCA (BIophysical ANalysis of Cell death and chromosome Aberrations), a biophysical model that simulates radiation-induced chromosome aberrations and cell death. More specifically, BIANCA was applied to produce a radiobiological database that predicts lymphocyte dicentric induction as a function of dose, particle type and LET. Afterwards, an interface with the FLUKA code allowed predicting dicentric RBE along a C-ion SOBP, and comparing it with cell survival RBE. This pilot study suggested that using the RBE for tumour cell survival along the entire beam might lead to an underestimation of the risk of normal tissue damage.

1. – Introduction

Chromosome aberrations are widely considered as radiation risk indicators (*e.g.*, for radiotherapy patients and astronauts [1]). In particular, some reciprocal translocations are thought to be causally related to specific tumor types [2-4]. Since the initial yield of translocations (that is, the yield immediately after irradiation) is basically equal to that

of dicentrics (*i.e.*, chromosomes with two centromeres resulting from incorrect rejoining of chromosome fragments deriving from two different chromosomes), in this modelling work we shall focus on dicentrics, for which more experimental data are available. More specifically, to calibrate the model parameter and to benchmark the model we considered *in vitro* data obtained by irradiating peripheral blood lymphocytes (PBL) with different radiation types at different LET values, and observing dicentric chromosomes at the first post-irradiation mitosis. Concerning the choice of PBL data, it is worth mentioning that translocations originated from hematopoietic progenitors/stem cells from bone marrow are a better cytogenetic biomarker for secondary cancer risk. However, at the moment, this information is not available in the literature for many different doses, radiation types and LET values, which is necessary to perform a systematic benchmarking of the model. Furthermore, the aim of this work, rather than quantifying the risk of normal tissue damage following hadrontherapy in absolute terms, consists in comparing the risk of secondary cancer following hadrontherapy with that following photon therapy by making use of a practical quantity that can be easily applied in clinics, that is the Relative Biological Effectiveness (RBE).

Dicentric chromosomes in peripheral blood lymphocytes (PBL) have many advantages: they are almost exclusively induced by ionizing radiation; they allow assessing individual whole-body exposures to doses as low as 0.1 Gy for low-LET radiation, thanks to their low background levels (typically, 0.001 dicentrics/cell); they are characterized by a close correspondence between *in vitro* and *in vivo* data. In addition to acute, whole-body exposure, dose estimation by dicentrics in PBL can also be achieved for protracted and/or partial-body exposure.

During radiotherapy, blood is unavoidably exposed, since lymphocytes circulate in the blood vessels and are distributed throughout the body. As a consequence, damage to the haematopoietic tissue is a major limiting factor with respect to the total dose that can be delivered in a radiotherapy treatment, due to both acute morbidity and the risk of developing secondary cancers [5].

In this framework, Durante *et al.* [6] monitored the induction of chromosomal aberrations in PBL of cancer patients treated with 10 MV X-rays or carbon ions (290 or 350 MeV/u spread-out Bragg peak) at NIRS in Chiba. The fraction of aberrant PBL was found to increase with the number of delivered dose fractions, reaching a plateau at high doses. Interestingly, while carbon ions were found to be more effective than equal doses of X-rays at inducing chromosomal aberrations *in vitro*, for the patients considered in that study the fraction of aberrant PBL was lower after carbon ion treatments than after X-ray treatments. This outcome was interpreted as proof of the improved dose distribution achieved with carbon ions.

It is also worth mentioning that chromosome aberrations in PBL are used for biodosimetry evaluations in astronauts, who are exposed to a mixed radiation field consisting of high-energy protons, He ions and heavier ions including iron. Pre-flight calibration curves are generally obtained exposing astronauts' blood samples to gamma rays *in vitro*. For each astronaut, the calibration curve is then used to convert the post-flight aberration yield into the equivalent dose. Reviews on this topic can be found in [7, 8].

In this framework, in the present work we applied the BIANCA biophysical model to create a radiobiological database able to predict the induction of dicentrics in lymphocytes as a function of dose, particle type and energy (and thus LET), within the ranges of interest for hadrontherapy treatments. Afterwards, BIANCA was interfaced to the FLUKA Monte Carlo radiation transport code to predict dicentric induction along a carbon ion beam, both in the entrance channel and at different positions along the

spread-out Bragg peak. The predicted RBE for dicentrics was then compared to RBE values for cell survival obtained in a previous work.

2. – Methods

The simulations performed in this work were based on the BIANCA biophysical model, which predicts the induction of chromosome aberrations and cell death by photons and by different ion types. BIANCA, which is implemented as a Monte Carlo simulation code, is based on the following main assumptions: i) ionizing radiation can induce "critical lesions" (CLs) of the DNA, where a CL is defined as a lesion that breaks the chromatin giving rise to two independent chromosome fragments; ii) distance-dependent mis-rejoining of such fragments, or fragment un-rejoining, produces chromosomal aberrations; iii) certain aberration categories (that is dicentrics, rings and large deletions, where "large" means visible when chromatin is condensed) lead to clonogenic cell death.

Since these assumptions have been discussed in detail in previous works [9-11], only a few key issues will be addressed here. In particular, the CL yield (*i.e.*, mean number of critical lesions per unit dose and unit DNA mass) is left as an adjustable parameter of the model, because the characteristics of the critical DNA lesions leading to chromosomal aberrations and cell death are still an open question in radiobiology [12]. The value of this parameter is strongly influenced by radiation quality (that is particle type and energy), although it also depends on the biological characteristics of the target cell. In general, the yield of CLs increases with LET (excluding very high LET values, where an overkill effect may occur), as well as with the cell radiosensitivity.

Like in our previous works on chromosome aberrations [13, 14], the distance dependence of the chromosome fragment rejoining probability was described by an exponentially decreasing function of the form $\exp(-r/r_0)$, where r is the initial distance between the free ends of the two fragments and r_0 is the characteristic distance of interaction that depends on the considered cell type. Based on previous results on lymphocytes [13], in this work r_0 was fixed to 0.8 μm. Further details on the model and the simulation techniques, including the way to take into account the important role of interphase nuclear architecture [15, 16], can be found in previous works [13, 14].

First of all, the CL yield was tuned by comparison with experimental data taken from the literature, more specifically with data on dicentrics in blood lymphocytes exposed to gamma rays and to monochromatic beams of protons, helium and carbon ions. For each ion type, the BIANCA simulations were performed for different LET values, and for each LET value the CL yield was adjusted *a posteriori*, following comparisons with the data.

For each considered ion type, the LET dependence of the CL yield (in terms of mean number of CLs per unit path length) was then analysed. For protons and carbon ions the CL yield was fitted using a linear-quadratic function, while for helium ions a linear function was found to be a better choice. For helium, the CL yields in the overkilling region, which were found to be lower than those in the lower LET region, were excluded by the fit, to avoid the risk of underestimating normal tissue damage. These parameterizations allowed to derive the CL yield to be used as a code input to predict dicentric induction at, in principle, any LET value, even where experimental data are not available.

A large number of dicentric dose-response curves (which were full predictions) was thus simulated for different particles and many different LET values. In particular, dose-response curves were simulated in the following LET intervals: 2.5–30 keV/μm for

protons, 5–110 keV/μm for helium ions, and 5–70 keV/μm for carbon ions, based on the LET range for which experimental data were available.

These (simulated) curves were then fitted by the familiar linear-quadratic function:

$$Y = \alpha D + \beta D^2, \tag{1}$$

and the α and β values were stored in a table as a function of ion type and LET, together with the linear and quadratic coefficients for gamma rays. This table, which constitutes a radiobiological database for dicentrics, was subsequently used for calculating the RBE for dicentrics along therapeutic-like carbon ion beams, exploiting a pre-existing interface between BIANCA and the FLUKA Monte Carlo transport code (version 2020.0, `www.fluka.org`).

For RBE evaluation, lithium, beryllium and boron ions were also considered in the database. In particular, the values of α and β for lithium were set equal to those for helium, while the values for beryllium and boron were set equal to those for carbon. This assumption was made necessary due to the lack of experimental data on dicentric induction by these ion types, for which therefore it was not possible to perform "*ad hoc*" simulations.

The α and β parameters stored in the table were for LET values up to 30.0, 110.0 and 70.0 keV/μm for protons, helium ions and carbon ions, respectively. For each ion type, the behavior at higher LET values was assumed to be described by the last available pair of α and β coefficients. This may imply an overestimation of the damage at very high LET values, in the so-called overkilling region. However, this choice was done to maintain a conservative approach, which is typical of radiation protection.

This radiobiological table was then read by FLUKA. More specifically, FLUKA calculated the necessary information (that is, particle type and energy, and absorbed dose) based on a voxel-by-voxel approach: when a certain amount of energy was absorbed in a voxel due to a given particle type of given energy (and thus given LET), FLUKA read the corresponding α_i and β_i values from the table and calculated the linear and quadratic coefficients for the mixed beam, based on the Theory of Dual Radiation Action, or TDRA [17], as described in [18], *i.e.*:

$$\alpha = \frac{\sum_{i=1}^{n} \alpha_i D_i}{\sum_{i=1}^{n} D_i}, \qquad \sqrt{\beta} = \frac{\sum_{i=1}^{n} \sqrt{\beta_i} D_i}{\sum_{i=1}^{n} D_i}. \tag{2}$$

Here, D_i is the absorbed dose (in the considered voxel) due to the i-th particle calculated by FLUKA, α_i and β_i are the corresponding radiobiological coefficients provided by BIANCA, and α and β are their average values considering that a mixed radiation field is present in the voxel.

The corresponding RBE in that voxel was calculated as D_X/D, where

$$D_X = \frac{-\alpha_X + \sqrt{\alpha_X^2 + 4\beta_X Y}}{2\beta_X}, \tag{3}$$

$$D = \frac{-\alpha + \sqrt{\alpha^2 + 4\beta Y}}{2\beta}. \tag{4}$$

In the formulae above, α_X and β_X are the photon coefficients provided by BIANCA, α and β are evaluated with eqs. (2), and Y is the dicentric yield.

3. – Results and discussion

3˙1. *Benchmark with experimental data.* – The initial phase of this work consisted of tuning the CL parameter by comparing BIANCA simulations with experimental data taken from the literature on dicentric induction in human lymphocytes irradiated by photons, considered as a reference radiation, or by different monochromatic ion beams.

The gamma-ray data reported in Schmid *et al.* [19] were considered for photons. The comparison between the data and BIANCA simulations is shown in fig. 1. The agreement was good for doses up to 3 Gy. At 4 Gy, the simulated dicentric yield was lower than the data. This may be explained by taking into account that, in experiments using Giemsa staining, some types of "complex exchanges" (defined as aberrations involving at least three chromosome breaks and at least two chromosomes), which play an important role only at higher doses, are often mis-classified as dicentrics.

The experimental data considered for protons were extracted from Rimpl *et al.* (LET = 3.5 keV/μm) [20], Edwards *et al.* (LET = 5.0 keV/μm) [21] and Schmid *et al.* (LET = 5.3 and 19.0 keV/μm) [22]. In fig. 1, a selection of these proton results is reported. A good agreement between calculations and data was found at 3.5 and 5 keV/μm, whereas at 19 keV/μm the simulated dicentric yields were higher than the observed ones at the higher doses. The reasons for this overestimation are still under investigation; however, the results can be considered as satisfactory given our interest in focusing on doses below 3 Gy, to stay within the range of therapeutic fractional doses.

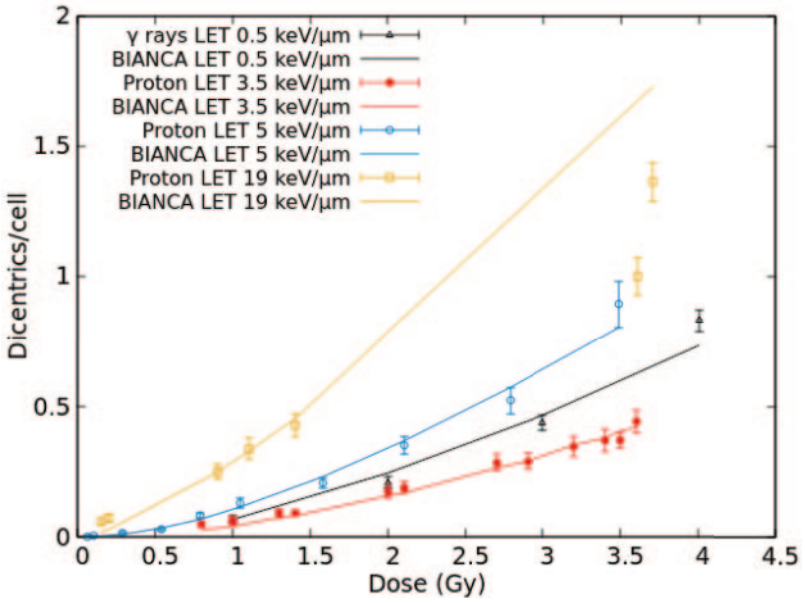

Fig. 1. – Mean number of dicentrics per cell as a function of dose induced by γ-rays of LET = 0.5 keV/μm and by protons having an LET of 3.5, 5 and 19 keV/μm. The points are experimental data, the lines are simulation outcomes.

Lymphocytes irradiated with protons of 3.5 keV/μm showed less dicentrics than those irradiated with gamma rays. This may be explained by considering that these data, although obtained by the same research group, were published in different papers (Schmid *et al.* for gamma rays [19], Rimpl *et al.* for protons [20]). Therefore, it is possible that the cells used in the two different experiments had a slightly different radiosensitivity.

For helium ions, the considered data were taken from Edwards *et al.* (^{3}He ions with LET = 22.0 keV/μm) [21], Edwards *et al.* (^{3}He ions with LET = 24.0 keV/μm and α-particles with LET = 140.0 keV/μm) [23], Di Giorgio *et al.* (^{4}He ions with LET = 31.4 keV/μm) [24], Greinert *et al.* (α-particles with LET = 113.0 keV/μm) [25], Schmid *et al.* (α-particles with LET = 150.0 keV/μm) [26] and Edwards *et al.* (α-particles with LET = 155.0 keV/μm) [27]. A selection of the results obtained for helium ions is reported in fig. 2. At 22.0 keV/μm, the simulations showed a tendency to underestimate the data at the lower doses and overestimate those at the higher doses. However, there was a good agreement for in the dose range between 1–1.5 Gy. For helium ions with LET = 31.4 keV/μm, the simulations reproduced the data quite well up to 2.5 Gy. At 3 Gy the simulated dicentric yield was higher than the observed one, but it is worth mentioning that this experimental point was lower with respect to the linear trend shown by the data at the lower doses. The dataset at 113.0 keV/μm consisted of nine experimental points: for each dose value, three points were obtained by observing the aberrations at 0, 8 or 14 hours after irradiation, using the Premature Chromosome Condensation (PCC) technique. These data were treated as a single data set as they followed the same fit, which was linear as it is typical for high-LET radiation. Also in this case, the simulations reproduced the data quite well.

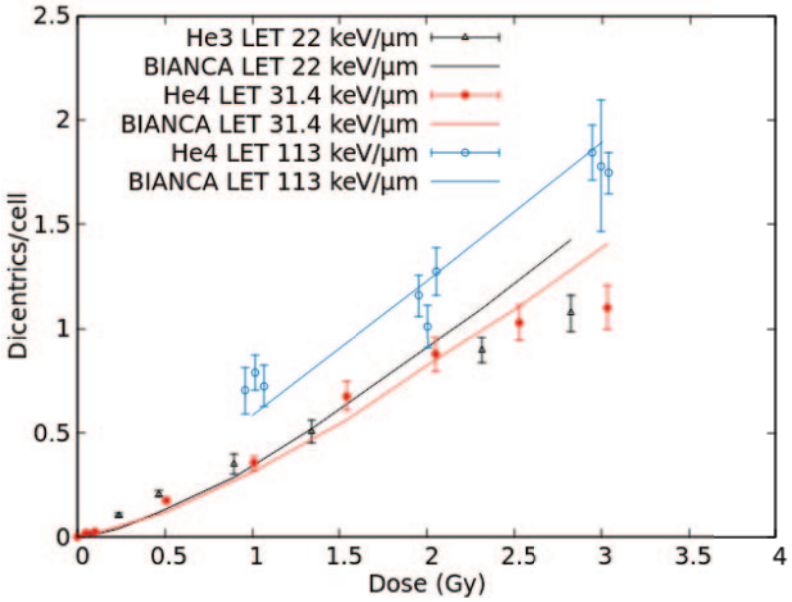

Fig. 2. – Mean number of dicentrics per cell as a function of dose induced by helium ions with LET = 22, 31.4 and 113 keV/μm. The points are experimental data, the lines are simulation outcomes.

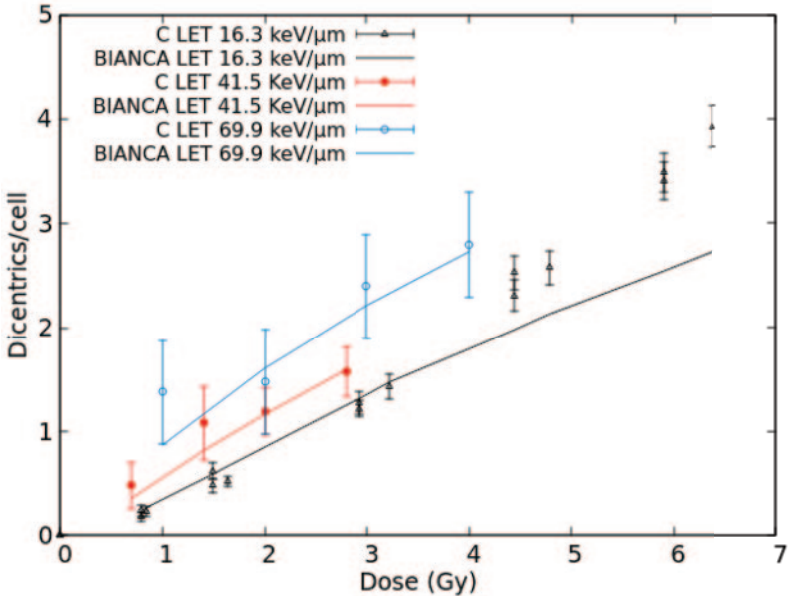

Fig. 3. – Mean number of dicentrics per cell as a function of dose induced by carbon ions with LET = 16.3, 41.5 and 69.9 keV/μm. The points are experimental data, the lines are simulation outcomes.

Concerning carbon ions, the experimental data for comparison were taken from Kowalska *et al.* (LET = 16.3 keV/μm) [28], Ohara *et al.* (LET = 22.4, 41.5 and 69.9 keV/μm) [29], Wang *et al.* (LET = 34.6 keV/μm) [30] and Di Giorgio *et al.* (LET = 61.0 keV/μm) [24]. In fig. 3, a selection of the results obtained for carbon ions is reported. At 16.3 keV/μm, the simulations reproduced the data up to about 3 Gy, whereas at higher doses the simulated dicentric yields were lower than the observed ones. Again, this may be due to the fact that, in the experiments, some complex exchanges may have been mis-scored as dicentrics. At 41.5 and 69.9 keV/μm, the agreement between experimental data and simulations was quite good.

Considering all the obtained results and taking into account that the goal of the present work was to focus on doses up to 3 Gy, since higher fractional doses are not of interest for cancer therapy, this work shows that BIANCA is able to reproduce the experimental data for all considered particle types and LET values.

3˙2. *Construction of the radiobiological database.* – To obtain the dose-response curves described above, for each ion type and each LET value the CL parameter was adjusted *a posteriori* following comparisons with experimental data. The LET dependence of the CL yield was then analysed for protons, helium ions and carbon ions, as shown in fig. 4.

For protons and carbon ions, the CL yield was found to be well fitted by a linear-quadratic function. In the case of helium ions, the CL yields found for LET values higher than 113 keV/μm, which were lower than the highest CL yield, were excluded from the fit to avoid to underestimate normal tissue damage. The remaining CL yields were found to be well fitted by a linear function. The fact that the last three CL yields were lower than the highest one can be explained by taking into account that they are in the so-called "overkilling region".

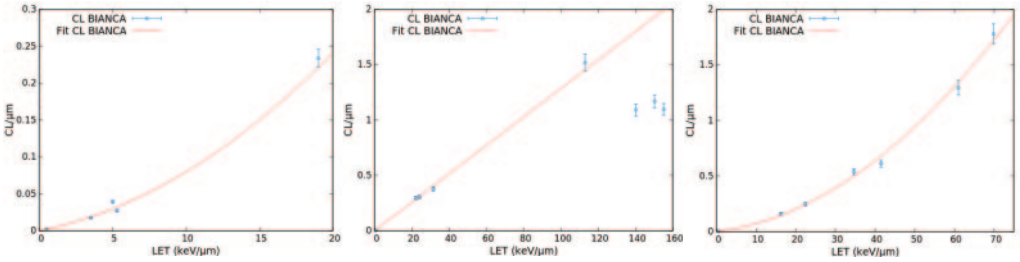

Fig. 4. – LET dependence of the CL yield (mean number of CLs per unit particle path length) for protons (left), helium ions (middle) and carbon ions (right).

Following this fitting procedure for the CL yield LET dependence, new BIANCA simulations were performed to obtain a large number of dose-response curves at many different LET values for monochromatic beams of protons, He ions and C ions. In fig. 5, a selection of these simulations, which are full predictions, is reported. For each given particle type and LET value, the dose dependence of the (simulated) dicentric yield was fitted by eq. (1), and the α and β parameters derived from the fit were stored in a table, that constitutes a radiobiological database for dicentric induction in lymphocytes.

3˙3. *RBE evaluation for a carbon SOBP.* – As explained above, the dose distribution for a carbon SOBP in water was calculated by FLUKA, and the variation of the dicentric RBE along the beam was calculated by reading the radiobiological database, thanks to the existing interface between BIANCA and FLUKA. In fig. 6, the distribution of the absorbed dose and of the RBE-weighted dose (D_{RBE}) are reported. In addition to the

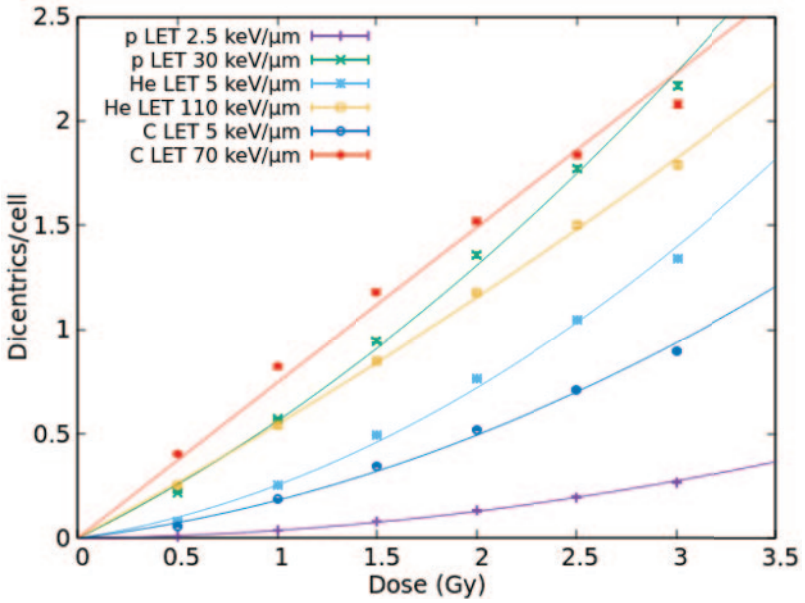

Fig. 5. – A selection of fully predicted dicentric dose-response curves for protons, helium ions and carbon ions.

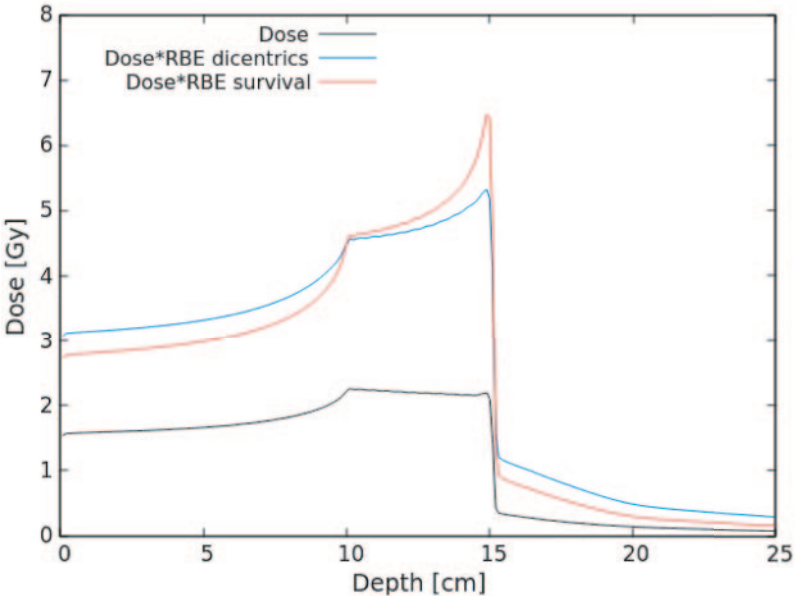

Fig. 6. – Distribution of absorbed dose and of RBE-weighted doses in a carbon SOBP.

RBE for dicentric induction obtained in the present work, the RBE for chordoma cell survival obtained in a previous work [31] was reported for comparison. Interestingly, in the entrance channel and in the fragmentation tail (which correspond to healthy tissue regions) the RBE for dicentrics was found to be higher than the RBE for cell survival. On the contrary, within the SOBP, the RBE-weighted dose for dicentrics was lower than that for cell survival. Furthermore, although both these quantities increased with depth until they reached a peak in the distal region, the peak of the RBE-weighted dose for dicentrics was lower than that for cell survival. This behavior may be explained by considering that the photon dose response for lymphocyte dicentrics is characterized by a lower linear coefficient and a higher quadratic coefficient with respect to the photon dose response for cell survival. As a consequence, at the lower (absorbed) doses, like in the entrance channel and in the fragmentation tail, the RBE for dicentrics is higher than that for cell survival, whereas at the higher doses, like in the SOBP, the opposite occurs. These results suggest that calculating the RBE in healthy tissues by adopting the same approach used for tumor cell killing might lead to an underestimation of the risk of late normal tissue damage, such as radiation-induced secondary tumors.

4. – Conclusions

In the framework of the estimation of normal tissue damage risk after hadrotherapy treatments, this work focused on the prediction of dicentric yields in human lymphocytes exposed to different radiation types by means of the BIANCA biophysical model. The benchmarking with experimental data taken from the literature showed that the model reproduces well the data for γ-rays and for different monochromatic beams of protons, helium ions and carbon ions, in the range of doses that is of interest for a typical hadrontherapy fraction (0–3 Gy). Fitting the LET dependence of the CL yield for each ion type

allowed simulating a large number of dicentric dose response curves at many different LET values, including those for which there are no experimental data. In turn, fitting these (simulated) curves by a linear-quadratic function allowed constructing a radiobiological database consisting of linear and quadratic coefficients that describe lymphocyte dicentric induction as a function of ion type and LET. This radiobiological database was then read by the FLUKA MC transport code, which allowed calculating distributions of absorbed dose and dicentric RBE-weighted dose along a carbon SOBP. A comparison with distributions of RBE-weighted dose for chordoma cell survival showed that the RBE for dicentrics was higher than the RBE for chordoma cell survival in the healthy tissue regions. If these results are confirmed, this would imply that considering only the RBE for tumor cell survival might lead to an underestimation of the risk of healthy tissue damage. As a future development, we plan to apply this approach to a certain number of patient cases, as well as to extend it to other tumor cell types and/or other beam configurations.

More generally, it is worth outlining that following this work BIANCA has the peculiarity of predicting both the beam effectiveness in tumor cell killing, and that in inducing normal tissue damage.

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