Reports of Practical Oncology and Radiotherapy 25 (2020) 68–78

Available online at www.sciencedirect.com

Reports of Practical Oncology and Radiotherapy

journal homepage: http://www.elsevier.com/locate/rpor

Original research article

Optimization of treatment planning for hypoxic tumours and re-modulation of radiation intensity in heavy-ion radiotherapy

Ladan Rezaee

Department of Physics, Shiraz Branch, Islamic Azad University, Shiraz, Iran

ARTICLE INFO

Article history:
Received 5 April 2019
Received in revised form
30 September 2019
Accepted 13 December 2019
Available online 17 December 2019

Keywords:
Hypoxic tumour
OER
Hadron therapy
Monte Carlo

ABSTRACT

Aim: The purpose of this study is to optimize treatment planning in carbon ion radiotherapy, taking into account the effect of tumour hypoxia.
Background: In conventional hadron therapy, the goal is to create a homogenous dose in the tumour area and, thus, achieve a uniform cell survival level. Since the induction of a specific damage to cells is directly influenced by the level of hypoxia in the tissue, the varying oxygen pressure in the different regions of hypoxic tumours would disrupt the uniformity of the cell survival level.
Materials and methods: Using the Geant4 Monte Carlo Code, the physical dose profile and dose-averaged linear energy transfer were calculated in the tumour. Then, the oxygen enhancement ratio in different areas of the tumour were compared with different pressures.
Results: Modulations of radiation intensities as well as energies of ion beams were calculated, both considering and disregarding the effect of hypoxia, and the required dose profiles were compared with each other. Cell survival levels were also compared between the two methods. An equation was obtained for re-modulating the beams in the presence of hypoxia, and radiation weighting factors were extracted for the beam intensities.
Conclusion: The results show that taking the effect of hypoxia into account would cause the reduction of average doses delivered to the tumour tissues up to 1.54 times. In this regard, the required dose is reduced by 1.63 times in the healthy tissues before the tumour. This will result in an effective protection of healthy tissues around the tumour.
© 2019 Greater Poland Cancer Centre. Published by Elsevier B.V. All rights reserved.

1. Introduction

Human tumour cells are not all under the same condition, but rather the inhomogeneity of their oxygen pressures would directly affect the response of each cell to ionizing radiation. The situation where tumour cells have low oxygen content (0.1–1 %) is called tumour hypoxia, and when the tumour tissue is completely depleted of oxygen (0 %), the tumour is said to be anoxic. On the other hand, tissues with high oxygen pressure (20 %) are called normoxic tissues, i.e. tissues that are under oxic conditions.

The reduced concentration of oxygen in the cells (hypoxic condition) results in a significant reduction in cell death rate after irradiation with ionizing radiation. This condition often occurs in clinical cases and is related to the inadequate supply of blood as compared with its consumption. This condition may occur for several reasons in minor areas within the tumour tissue and may have different impacts, but this is generally related to poor treatment outcomes in radiotherapy.[1]

The efficacy of hypoxia treatment depends strongly on the linear energy transfer (LET) of irradiated particles.[2] In fact, it is only through relatively high-LET radiation that we can reduce the effects of hypoxia and increase the efficacy of our treatment. Therefore, with adequate information on the spatial distribution of different hypoxia levels within a tumour (i.e. different areas with different oxygen pressures) and the relationship between radiation resistance and LET, we can optimize the applications of different particles.[3] Even within a tumour, the ratio of radiation doses required for adjacent hypoxic and normoxic regions is similar to the ratio of radiation doses required for a hypoxic tumour and adjacent healthy tissues.[4,5] Obviously, photons are not a good candidate for this type of treatment, and heavy ions, due to their specific physical and radiobiological characteristics,[6] are a more suitable choice in order to allow steep dose gradients between hypoxic and normoxic sections within the tumour.[7]

Recently, the study of the properties of hypoxic tumours and attempts to optimize treatment methods have expanded in the lit-

E-mail address: Ladanrezaee313@gmail.com

https://doi.org/10.1016/j.rpor.2019.12.014
1507-1367/© 2019 Greater Poland Cancer Centre. Published by Elsevier B.V. All rights reserved.

erature both from a theoretical and empirical point of view. In this regard, research has been carried out to determine the exact oxygen concentration in a given target[8] and provide a standard method for measuring oxygen pressure under *in vivo* conditions.[9] Efforts have also been made to employ tissue imaging in order to determine oxygen distribution and concentration.[10–13] Furthermore, the studies conducted by Lin et al.[14] and Toma-Dasu et al.[15] can also be pointed out in connection to the relationship between oxygen uptake and content.

In general, the problem of adjusting the radiation dose for each section of the tissue, based on its oxygen content, has been investigated in several ways. In this regard, Toma-Dasu et al.,[15] Toma-Dasu et al.,[16] Sovik et al.,[7] Sovik et al.,[17] and Sovik et al.[18] can be pointed out. The 'dose painting'[19–21] and the later 'LET painting'[22] techniques were also proposed in this relation. Furthermore, the 'kill-painting' technique was also developed later on.[23]

Among the experimental studies in this area, we can mention Furusawa et al.[24] In that study, HG, V79, and T1 cell lines were irradiated with energetic ions of ^{20}Ne, ^{12}C and ^{3}He under both aerobic and hypoxic conditions and experimental data were collected based on a number of parameters. Moreover, in Tinganelli et al.,[25] Tinganelli et al.[26] and Durante et al.,[27] multiple cell lines were irradiated with various energetic ions under all three aerobic, hypoxic, and anoxic conditions. Other experimental studies have also been carried out in this area, a full review of which can be found in Wenzl and Wilkens[28] and Stewart et al.[29] In addition, a review of the photon studies on this subject can be found in Sovik et al.[7,17]

The dependence of oxygen enhancement ratio (OER) on various parameters has been investigated in different studies. In this respect, the dependence of OER values on LET has been studied by Wenzl and Wilkens,[28] Stewart et al.,[29] Scifoni et al.,[30] Antonovic et al.[31] These studies predicted a severe reduction in OER values at LETs higher than 100 keV/μm. The correlation between OER values and RBE, both of which are LET-dependent is investigated in Blakely et al.[32] Moreover, there have also been investigations into the dependence of OER on survival,[30,33] radiation dose,[24,34,35] tissue type,[26,35,36] and ion type.[24]

There has also been a number of theoretical, quasi-experimental, and simulation studies in the field. For example, the study by Refs.[28,35] comes to mind, in which a simple model for acquiring the OER was developed by collecting and categorizing experimental data from other literature. Their proposed model incorporated the two standard linear-quadratic (LQ) and Alper-Howard-Flanders (AHF) models. In Scifoni et al.,[30] a simple semi-empirical formula was used to calculate the OER as a function of mean oxygen concentration and LET. Furthermore, Malinen and Søvik[5] compared the outcomes of dose-painting and LET-painting methods in tumour control. In Sokol et al.,[37] experimental data were used to study the oxygen ion irradiation of hypoxic tumours.

In this regard, the TRiP98 (Treatment planning for particles) code[38–41] which has excellent possibilities in treatment planning, and then TRiP-OER[23,30,42] were expanded.

In the modulation of radiation intensity in conventional irradiation, which is done with no regard to tumour hypoxia, biological dose is kept constant throughout the tumour. But since the areas with a higher level of hypoxia exhibit higher radiation resistance, this method prevents uniform damage to the entire tumour. Thereby, radiation intensity modulation through the method used in conventional radiotherapy would not yield favorable results.

2. Aim

The main aim of the present work is to design the deliverance of uniform biological damage in a hypoxic tumour with varying oxygen pressures in different regions within the tumour. To overcome the radiation resistance that occurs in hypoxic tissues, we need a relatively high LET radiation. Even in the case of heavy ions, such as carbon, this situation occurs only in small portions of an extended target irradiation (i.e. close to the distal end).[30] The number of lethal lesions in the tumour cell nucleus, which results in cell inactivation, is proportional to the dose delivered to the cell nucleus.[43] On the other hand, decreasing oxygen concentration in tumour cells increases the probability of successful repair of these lethal damages.[44] Therefore, for an optimal treatment, having the spatial distribution of different levels of hypoxia in the tumour, as well as the dependence of radiation resistance on different LETs in each segment of the hypoxic tissue, it is necessary to optimize the ion type, projectile energy, and ion beam intensity. The main purpose of this paper is to optimize the latest case, namely the intensity of the ion beam. Hence, the method presented in this paper can be used for any other heavy ion type. Whichever type of ion is selected, the energy of the landing particles must be adjusted to cover the spatial range of the tumour area. In performing the Monte Carlo calculations carried out here by the Geant4 code,[45] it is possible to calculate the dose and LET values for each main ion (with arbitrarily positive charge) at any depth within the phantom (with an arbitrary chemical composition). Hence, the specific ion type and particular cell line considered in this article are just an example. Thus, one way to solve the hypoxia problem may be to increase the dose delivered to the cell nucleus, and such a choice seems to keep the error within an acceptable range.

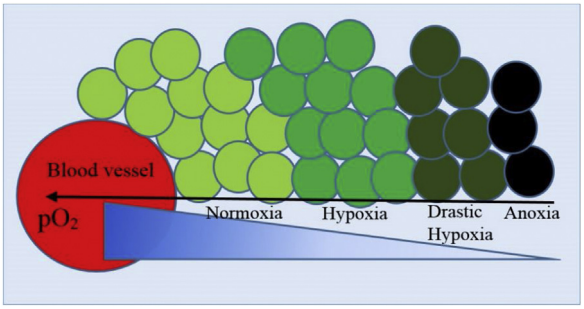

Fig. 1. Inhomogeneity in oxygen content in the tissue of a hypoxic tumour.

In this paper, beam intensity is modulated based on tumour hypoxia conditions. To calculate the OER, the model presented in Refs.[28,35] was employed. Using Geant4, a Monte Carlo simulation of ^{12}C ion irradiation was carried out to calculate the absorbed dose profile and dose-averaged LET. Furthermore, to determine the biological effects of radiation on tissues, taking the CHO-K1 cell line into account, RBE values were calculated for ^{12}C ion irradiation with the aim to assess the biological dose. The modulation was done based on RBE and OER values at various depths within the tumour, such that more severely hypoxic areas received higher doses. This modulation is based on matrix calculations, as will be described in the following section. Moreover, the proposed method was applied to a case of a hypoxic tumour with variable oxygen pressures, and the results were presented.

In this study, the intention was not to focus on a specific type of ion, and the method can also be applied to protons and other heavy ions.

3. Materials and methods

Fig. 1 shows a schematic view of the different parts of a hypoxic tumour. Anoxic cells are already dead, and the normoxic cells that are adjacent to capillaries proliferate at an extremely high rate. However, due to the high oxygen content in this area, the normoxic cells have no resistance against radiation damage and are

destroyed. The most dangerous part of the tumour consists of hypoxic cells that are resistant to radiation. This effect is originated by a reduction in the mechanisms of DNA damage, which is mediated by free radicals. In the presence of oxygen, biological damage to DNA is stabilized and sustained.[46]

The radiation resistance produced by hypoxia is quantitated by the oxygen enhancement ratio (OER), which is the ratio of radiation doses required to produce the same biological effect at a given (low) oxygen concentration and under highly oxic conditions (air), i.e.,

$$OER(pO_2) = \left.\frac{D(pO_2)}{D_a}\right|_{same\ effect} \quad (1)$$

where pO_2 is the partial pressure of oxygen, D_a is the dose under normaxic conditions ($pO_2 = p_a \sim 30$ mmHg), and $D(pO_2)$ is the dose required to achieve the same effect under hypoxic conditions with $pO_2 < p_a$.

The dependence of cellular survival (S) on delivered dose (D) is expressed through the linear-quadratic model LQ as follows:

$$S = \exp(-\alpha D - \beta D^2) \quad (2)$$

where α and β are two parameters that have been parameterized in terms of LET and describing the linear and quadratic parts of the survival curve, respectively. The α/β ratio is related to the tissue and radiation types. The dependence of these two parameters on oxygen pressure and LET is determined via the following equations[28,35]:

$$\alpha(L,p) = \frac{(a_1 + a_2 L)p + (a_3 + a_4 L)K}{p + K} \quad (3)$$

$$\sqrt{\beta(L,p)} = \frac{b_1 p + b_2 K}{p + K} \quad (4)$$

where p is the partial oxygen pressure (mmHg) in a given section of the tumour, L is equal to the LET value (keV/μm), and K is the parameter controlling the trend of OER alterations based on pO_2. The parameters a_1, a_2, a_3, a_4, b_1, and b_2 are obtained through a graph fitting to experimental data. In Refs.,[28,35] the considered values were K =3.0 mmHg, $a_1 = 0.22\ Gy^{-1}$, $a_2 = 0.0024\ \mu m(Gy\ keV)^{-1}$, $a_3 = 0.050\ Gy^{-1}$, $a_4 = 0.0031\ \mu m(Gy\ keV)^{-1}$, $b_1 = 0.40\ Gy^{-1}$, and $b_2 = 0.15\ Gy^{-1}$, which refer to the experimental data available for all cell types and types of radiation. Thus, according to Wenzl & Wilkens (2011), the OER value is obtained from the following equation:

$$OER = \frac{\sqrt{\alpha^2(L,p_h) - 4\beta(L,p_h)\ln(S)} - \alpha(L,p_h)}{\sqrt{\alpha^2(L,p_a) - 4\beta(L,p_a)\ln(S)} - \alpha(L,p_a)} \cdot \frac{\beta(L,p_a)}{\beta(L,p_h)} \quad (5)$$

where S is the survival fraction, and p_a and p_h stand for the partial oxygen pressure under aerobic and hypoxic conditions, respectively ($p_h \le p_a$). Since the survival fraction is dose-dependent, the OER will be dose-dependent as well. Using this equation, we can calculate the OER for each pO_2 value under both oxic and hypoxic conditions.

The relative biological effectiveness (RBE) is defined as the ratio of the absorbed dose of the reference radiation (photon) to the absorbed dose of the radiation under study, provided that both types of radiation exhibit the same biological effect on the tissue cells. Thereby,

$$RBE = \left.\frac{D_{ph}}{D}\right|_{isoeffect} \quad (6)$$

where D_{ph} and D are the photon dose and the dose of the radiation under study, respectively. Having obtained the values of α and β, the RBE can be calculated using the following equation[47]:

$$RBE = \frac{-1}{2D}\left(\frac{\alpha_{ph}}{\beta_{ph}}\right) + \frac{1}{D}\sqrt{\frac{1}{4}\left(\frac{\alpha_{ph}}{\beta_{ph}}\right)^2 + \left(\frac{\alpha_{ion}}{\alpha_{ph}}\right)\left(\frac{\alpha_{ph}}{\beta_{ph}}\right)D + \left(\frac{\beta_{ion}}{\beta_{ph}}\right)D^2} \quad (7)$$

where α_{ion}, β_{ion}, α_{ph} and β_{ph} are the LQ parameters of the response to both ions and the reference radiation.

Disregarding the effect of OER on hypoxic tissues, the biological dose (D_{bio}) at any given point within the tissue (x) is obtained by multiplying the physical dose by the RBE value at that point:

$$D_{bio}(x) = D_{phy}(x).RBE(x) \quad (8)$$

Therefore, to create a homogeneous biological dose in the tumour in which the oxygen conditions are uniform (e.g. the entire tumour being under oxic conditions), radiation intensity should be modulated in such a way that D_{bio} remains constant throughout the tumour.

If we denote the weighting factor for the intensity of the jth beam by wj, then the homogeneous dose at each point within the tumour is obtained as:

$$D_{Tumor}(x) = \sum_{j=1}^{M} w_j . D_j(x) \quad (9)$$

where M is the number of weighted beams, which is equal to the number of Bragg peaks within the tumour. $D_j(x)$ is the dose corresponding to the jth beam at the position x, which can be either the physical dose (expressed in Gy) or the biological dose (expressed in Gy(RBE)). So, if we want to modulate the radiation intensity with no regard to the effect of OER, Eq. (9) is used according to the conventional treatment planning.

Beam intensity modulation is, technically, the calculation of weighting factors. We use the method previously presented in Rezaee[48] for this purpose. This method is based on matrix calculations using the absorbed dose profiles derived from Monte Carlo calculations.

The reason for using matrix calculations is to determine coefficients that can calculate the appropriate dose at any point in the tumour range. For this purpose, the following parameters are considered in these calculations:

(1) Radiation ion energy for creating a Bragg peak at every point within the tumour.
(2) RBE value at each point within the tumour (In this case, the biological dose required to cause the same damage throughout the tumour can be evaluated. Therefore, at this stage, the cell line type is needed to determine the coefficients of the LQ model, as well as the LET value at each point in the tumour).
(3) OER value at the target point (In this case, based on the effect of hypoxia, the dose can be adjusted in order to design the radiation intensity in a way that the oxygen-deficient spots receive the appropriate dose in terms of oxygen concentration. This is done directly, according to the tumour hypoxia maps and the oxygen concentration at each point within the tumour).

In this regard, using Monte Carlo calculations (via Geant4 in this paper), the absorbed dose and LET values are obtained. Then, using an algorithm of matrix computations, the weighting factors corresponding to the intensity of each ion beam are calculated taking into account the three abovementioned parameters. Obviously, including these parameters in the determination of dose distribution would lead to a non-uniform absorbed dose across the tumour area.

After calculating the absorbed dose at each point in the tumour, we define a $M \times M$ matrix called D, where the element $D_{i,j}$ represents the dose value (in Gray) delivered by the beam with energy E_j at location x_i (normalized dose per particle). The locations x_1 through x_M are the exact locations of the Bragg peaks extracted from the Monte Carlo calculations and are all within the desired spread-out Bragg peak (SOBP) range.

Determining the weighting factors wj for the intensity of the j-beam with the specified energy means that by multiplying each factor wj in the j-column of the matrix D, the sum of the elements of each row of the matrix equals an appropriate dose value, which results in the same biological effect in the tumour range. So, we have for every row of matrix D:

$$\begin{cases} w_1 D_{1,1} + w_2 D_{1,2} + \ldots + w_M D_{1,M} = d_1 \\ \vdots \\ w_1 D_{M,1} + w_2 D_{M,2} + \ldots + w_M D_{M,M} = d_M \end{cases} \tag{10}$$

where d_1 to d_M is the appropriate dose for producing the same biological effect across the tumour area, taking into account the biological characteristics of each point (by RBE), as well as the environmental oxygenation in them (by OER). These values will be obtained in the following equation.

By defining a weighting factor matrix denoted by W with the dimensions $1 \times M$, we can summarize the equation set (10) in the following matrix multiplication:

$$(w_1, w_2, \ldots, w_M)_{1\times M} \begin{pmatrix} D_{1,1} & \ldots & D_{M,1} \\ \vdots & \vdots & \vdots \\ D_{1,M} & \ldots & D_{M,M} \end{pmatrix}_{M\times M} = (d_1, d_2, \ldots, d_M)_{1\times M} \tag{11}$$

The first side of the expression is the matrix multiplication of W in the transpose matrix of D, i.e. D^T. Therefore, by introducing the matrix d, which is a $1 \times M$ matrix and its elements are equal to d_1 to d_M, we will have a matrix equation as follows, in which the elements of the matrix W are our unknowns[48]:

$$W(D)^T = \mathrm{d} \tag{12}$$

Obviously, by multiplying both sides of this equation by the inverse of the matrix D^T from the right, the elements of the matrix W can be calculated:

$$W = \mathrm{d}\left(D^T\right)^{-1} \tag{13}$$

Therefore, the value of each weighting factor is obtained as follows:

$$w_j = \sum_{i=1}^{i=M} d_i ((D^T)^{-1})_{i,j} \tag{14}$$

Thus, the weighting factors of intensity are calculated for M ion beams. To have a uniform absorbed dose distribution, all we need to do is to calculate the weighting factors from the equations above for the same d_i values (for i = 1, . . ., M), where each d_i is equal to the arbitrary maximum dose of d_{Max} in the whole tumour area. In this case, the weighting factors are:

$$w_j = d_{Max} \sum_{i=1}^{i=M} ((D^T)^{-1})_{i,j} \tag{15}$$

Now, to apply the RBE and OER values in the calculation of weighting factors, all we need to do is to consider the elements of the matrix d properly. The biological dose at each point is obtained according to Eq. 8. In order to achieve a uniform biological effect within the tumour, given the amount of OER at each point, sites with lower oxygen concentrations would require higher doses. So, again, by choosing the desired dose value d_{Max} in the entire tumour area, the values of the elements in matrix d will be as follows:

$$d_i = \frac{d_{Max}}{OER(x_i)} \tag{16}$$

Now, we determine the relation between the weighting factors without considering the effect of OER (which we call w_j) and considering this effect (which we call $w_j^{'}$). If we were to modulate the radiation beams in accordance with the effect of OER, the dose required to create a homogeneous cell survival in the entire tumour would no longer be fixed. Hence, new weighting factors should be calculated so that more severely hypoxic regions receive higher doses according to their OER. Therefore, Eq. (9) should be modified as follows:

$$D_{Tumor}(x) = \sum_{j=1}^{M} w_j . [OER_j(x) . D_j(x)] \tag{17}$$

where $OER_j(x)$ is the OER corresponding to the jth beam at the position x. In order to count in the effect of OER in the weighting factors, Eq. (17) must be reformulated in the form of Eq. (9) with new weighting factors that we introduce in the following. To this end, by integrating both sides of Eq. (17) over the variable x from a to x, where a is a constant, we have:

$$\int_a^x D_{Tumor}(x)dx = \sum_{j=1}^{M} w_j . \int_a^x OER_j(x) . D_j(x)dx \tag{18}$$

Next, after multiplying and dividing the right side of Eq. (18) by $\int_a^x D_j(x)dx$ and defining the mean value of OER through the following equation:

$$\left\langle OER_j \right\rangle_D = \frac{1}{\int_a^x D_j(x)dx} \int_a^x OER_j(x) . D_j(x)dx \tag{19}$$

Eq. (18) can be reformulated as follows:

$$\int_a^x D_{Tumor}(x)dx = \int_a^x \sum_{j=1}^{M} w_j . \left\langle OER_j \right\rangle_D . D_j(x)dx \tag{20}$$

Since the dose function is continuous, by differentiating both sides of the previous equation in terms of x, we will have:

$$D_{Tumor}(x) = \sum_{j=1}^{M} \left(w_j . \left\langle OER_j \right\rangle_D \right) . D_j(x) \tag{21}$$

Therefore, the new weighting factors for beam intensity will be as follows, taking into account the effect of OER:

$$w_j^{'} = w_j . \left\langle OER_j \right\rangle_D \tag{22}$$

where Eq. (9) is reproduced again. These weighting factors cause a non-homogeneous dose distribution within the hypoxic tumour in accordance with the severity of hypoxia in each region in order to produce the same biological effect across the entire tumour tissue.

Therapeutic gain, simply known as TG, is defined as the ratio of average radiation dose in conventional treatment to average radiation dose based on the effect of tumour hypoxia.[5] The conventional treatment design is defined as treatment with a variety of ions, which leads to a homogeneous dose distribution throughout the tumour. Furthermore, in treatment with consideration to the effect of hypoxia, the average dose in the tumour equals the mean of radiation doses in each area within the tumour. The dose required for each area is calculated by modulating the radiation

Table 1
Experimental value of the parameters of the LQ model in the X-ray reference radiation and carbon radiation (with LET = 100 keV/μm) for the CHO-K1 cell line.[26]

	$\alpha_{ph}(Gy^{-1})$	$\beta_{ph}(Gy^{-2})$	$\alpha_{ph}(Gy^{-1})$
Oxic	0.810 ± 0.012	0.020 ± 0.001	0.164 ± 0.006
Hypoxic	0.639 ± 0.042	0.0079 ± 0.0010	0.140 ± 0.017
Anoxic	0.409 ± 0.024	0.0027 ± 0.0005	0.089 ± 0.01

intensity according to the OER in that area, and the aim is to produce a uniform biological effect throughout the tumour with emphasis on a potential reduction in the overall dose received by the tumour. Therefore,

$$TG = \left.\frac{\bar{D}_C}{\bar{D}_H}\right|_{same\ effect} \tag{23}$$

where $\bar{D}_C$ and $\bar{D}_H$ are the average doses in conventional treatment and treatment with consideration to tumour hypoxia, respectively. Since there is a lower average dose required when hypoxia is taken into account, the therapeutic gain would be greater than 1.

The CHO-K1 cell line belongs to Hamster ovary cells. Experimental data on the values of α_{ph} and β_{ph} in the reference radiation, for which X-rays are selected in this context, were extracted from Tinganelli (2013). These values are provided in Table 1 under three conditions: oxic, hypoxic and anoxic.

Oxygen pressure was considered as $p_a = 30$ mmHg under oxic conditions. The clinical values for oxygen pressure under hypoxic conditions (p_h) are usually considered within the range of 0.5−20 mmHg. Although, in the case of drastic hypoxia, oxygen pressure is considered equal to 0.01 mmHg. In a clinical treatment planning, which requires maximum precision, we must have access to an accurate map of the tumour hypoxia that has the oxygen concentration specified in it geometrically, point by point. To do this, we need to have a method to accurately determine the oxygen concentration in the tissue. One method that makes this possible is functional magnetic resonance imaging (f-MRI).[13] After determining the spatial distribution of different hypoxic levels in the target, while knowing the radio-resistance in different LETs, we need to modulate the intensity of the ion beam used to target each and every point within the tumour based on the oxygen concentration at each point. The modulation of ion beam intensities, as well as the modulation of energy, which determines the location of the Bragg peak at any point in the tumour, optimizes the treatment system. In this paper, the modulation of intensity is investigated.

Calculating the OER at various points within the phantom based on different hypoxia conditions would provide us with a proper criterion for calculating the doses required to produce an identical biologic effect across the tumour. To design a SOBP that would cover the entire tumour tissue, we need dose-averaged OER values ($\bar{OER}(x)$) for all radiation types, which are obtained through the following equation:

$$\bar{OER}(x) = \frac{\sum_{i=1}^{N} OER_i(x) D_i(x)}{\sum_{i=1}^{N} D_i(x)} \tag{24}$$

where N indicates the total number of beams, and $OER_i(x)$ and $D_i(x)$ denote the OER and received dose corresponding to the ith beam at the position x, respectively. Therefore, having obtained the dose and LET values for all points through the Geant4 code, OER values can be calculated for different radiation types at various depths within the phantom. To this end, the α and β values are first calculated for each radiation type at a given oxygen pressure using Eq.s (3) and (4), and then, Eq. (5) is solved based on those values.

In this study, we use the Geant4 Monte Carlo simulation toolkit to simulate cases of ion beam radiation therapy aiming to calculate the required dose and dose-averaged LET for water phantoms. For this purpose, we use the Geant4 hadrontherapy example presented in Cirrone et al.[49] The output data collected from the simulation were based on every 0.1 *mm* of the phantom depth. The selected incident beam was a pencil beam 2 *mm* in radius without any divergence. Using the Geant4 code, the dose profiles and depth-dependent dose-averaged LETs were extracted. In these simulations, a cubic water phantom with 400-mm-long sides was considered and carbon ions were irradiated into one of the cube faces in the form of pencil beams. Furthermore, a hypothetical tumour with a width of 50 mm was considered, the center of which was located at a depth of 65 mm within the phantom (These dimensions are arbitrarily selected and the energy of the ions is adjusted so that the location of the Bragg peak would be within the tumour range).

To design an SOBP, the depth dose distribution for carbon beams with different energies must be calculated, as well as LETs. The Geant4 computational code is a complete tool for such computations.[45] QGSP_BIC_EMY is the best physics models in the hadrontherapy program in this code. Therefore, the hadronic interactions for nucleons are defined by the quark-gluon string pre-compound model (QGSP). The binary ion cascade model (BIC) simulates inelastic interactions for ions and the electromagnetic Y model (EMY) simulates electromagnetic interactions between all particles.[49] In the execution of the program, the transportation of twenty million radiation particles were simulated.

In this paper, we have used the hadrontherapy example from Ref.[49] to calculate the absorbed dose distribution, as well as the dose-averaged LET. In the latest version of this example, specific features and tools have been added that allow for an error-free activation of the available physical models.[50,51] The potential of these new aspects was simulated for proton and carbon ions in LNS-INFN, commonly used for radiobiological experiments. The results show that the method used in the hadrontherapy example would guarantee the best quality in the final results.[51]

4. Results

In this paper, radiation intensity modulation was performed only on the main axis of the tumour. Technically, to cover the entire geometry of any given tumour with a specific geometry, an ion beam must be applied to each specific axis of the tumour to target a specific part of the tumour. In this case, each ion beam must be modulated separately. The distance between the pathways of major ions must be selected in a way that all tumour cells get affected, whether located on the major axis of radiation or within the spaces in between the major ion pathways. Since the majority of the secondary electrons in the tissue are losing most of their energy within 1–1.5 nm of major ion pathways,[52] if the distance between the pathways is about that long, the modulated computations in this article will be applicable. Most experimental measurements in the survival curve calculation have been done in vitro, and the applied doses correspond to what is used during treatment. These doses are not that high, meaning that the heavy ion fluxes are small enough for the ionic pathways to not overlap.[53] Since the radiation dose is proportional to the flux and LET of the irradiated ions, the distance between ionic pathways can be calculated by knowing the dose and LET in any particular problem.[53]

Since carbon ions are high-LET beams, they are selected as radiation particles in this study. A total of 24 carbon ion beams with energies ranging from E = 132 MeV/u to E =207 MeV/u were tracked along the main axis of the tumour using the Geant4 simulation code. To create a spread-out Bragg peak throughout the tumour,

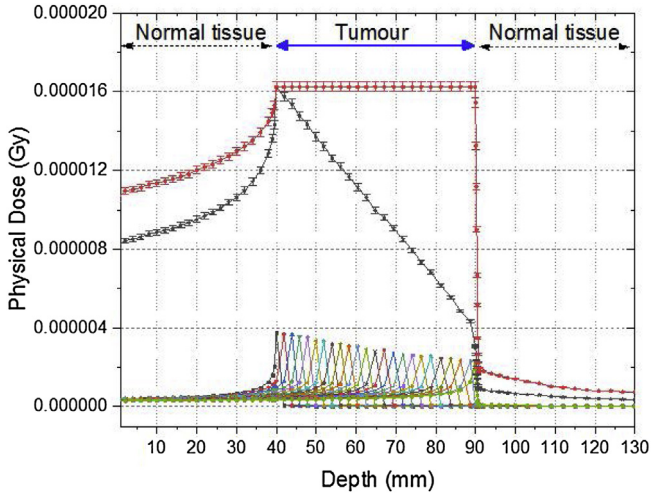

Fig. 2. Physical absorbed dose profiles resulting from 24 carbon ion beams with energies ranging from E = 132 MeV/u to E = 207 MeV/u using the Geant4 code. The Bragg peaks of these beams were located at a depth of x = 40 mm to x = 90 mm. Total dose without beam intensity modulation (black curve) as well as with modulated beam intensities (D_{Tumour}) from Eq. 9 (red curve) are also represented.

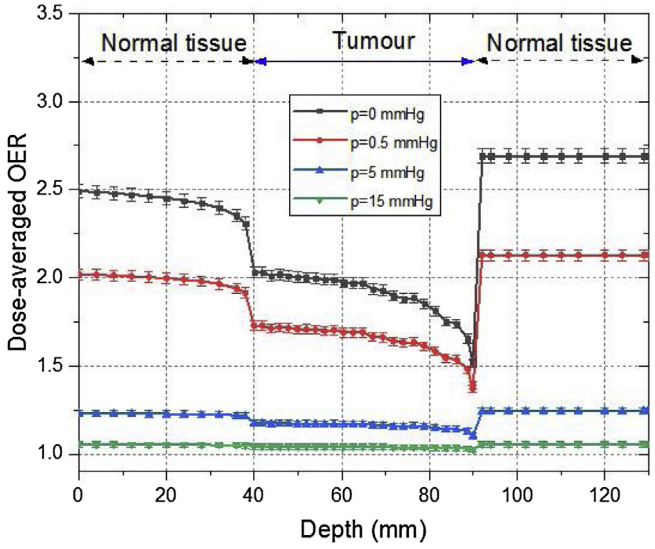

Fig. 3. The changes in dose-averaged OER based on depth for 4 different oxygen pressures that is assumed to be constant throughout the entire tumour tissue. The dose-averaged OER was calculated by Eq. 18. The dose corresponding to the *i*th beam at the position *x* was calculated by Geant4 Code.

the energies of ion beams were adjusted in a way that the Bragg peak corresponding to the beam with the lowest energy was at a depth of x = 40 mm, and the one corresponding to the beam with the highest energy was located at x = 90 mm. The energy difference between each two consecutive beams equaled 3.34 MeV/u and the equivalent distance between their Bragg peaks was about 2 mm. Fig. 2 illustrates the physical absorbed dose profiles resulting from these beams, as well as their sum total. In the Geant4 simulation, the reproduced physical dose distribution is measured at a maximum error of 3 % in the normalized physical dose.[54] In our calculations, despite the high number of particles in the simulation (20 million particles), and despite using the hadrontherapy example[49] in which the computational quality is very high, the maximum relative error was 1.5 % in the physical dose calculations, as well as the dose-averaged LET.

Fig. 3 shows the changes in OER based on depth for 4 different oxygen pressures, all within the clinical range of hypoxia. In this figure, oxygen pressure is assumed to be constant throughout the entire tumour tissue. To do this, we first calculate the alpha and beta values for 24 different beams of carbon ion radiation (the physical absorbed dose profiles of which are shown in Fig. 2), having obtained the LET values by running Geant4 for each beam. Hence, using Eq. 3 and 4, taking into account a given oxygen pressure value, for each point in the hypothetical tumour area, the alpha and beta values of each beam were calculated in terms of depth within the phantom. Here, oxygen pressures of p = 0 mmHg, p = 0.5 mmHg, p =5 mmHg, and p = 10 mmHg were considered, which are within the range of hypoxic oxygen concentration, as well as the oxygen pressure of p = 20 for normoxic tissues. Then, using Eq. 5, the OER value at each point was calculated considering a 10 % survival level (S = 0.1) for each ion beam in terms of depth. Next, using Eq. 24, the dose-averaged OER was calculated. To do this, having the dose value of each beam at each point (from the Geant4 output shown in Fig. 2), multiplying it by the OER value of that beam at that point, adding all these values together, and then dividing the result by the total dose of all beams at that point, the dose-averaged OER value is obtained at each point in the tissue area. These calculations were repeated for 24 ion beams, as well as for 5 different oxygen values, and the results are plotted as a function of depth in Fig. 3 for the dose-averaged OER.

As can be observed, OER increases as oxygen content decreases in the tissue. Also, at a specific pressure, OER decreases as we go deeper into the tumour. This means that with the same hypoxia condition in the entire tumour, it will be possible to produce the same amount of damage in tumour cells within deeper regions, as compared with cells in shallower depths, using a lower radiation dose. This result is of critical importance as it is in contrast with conventional heavy-ion radiation therapy.[6,38,42,55] In conventional treatment planning, which is done with no regard to the effect of OER on tumour cells, the goal is to achieve a homogeneous dose throughout the tumour, and beam intensity is modulated based on that objective. However, this view would change if we were to consider the influence of OER.

In clinical cases, oxygen pressure is not constant over the entire tumour tissue. In this regard, the more central points of the tumour are under more drastic hypoxia conditions. Fig. 4a shows the geometric design of a hypoxic tumour within a healthy tissue. Oxygen pressure was considered to be 30 mmHg in both the healthy tissue and the normoxic region of the tumour. The hypoxic part of the tumour was divided into several regions with various oxygen pressures ranging from 0.01 mmHg to 20 mmHg, and specific widths were determined for each region. Furthermore, the anoxic region was characterized by $pO_2 = 0$. Fig. 4b shows the dose-averaged OER in this proposed hypoxic tumour. The dose-averaged OER values vary between one and two. In the drastic hypoxia region, the OER value is the highest. This calls for very steep dose-averaged OER gradients between hypoxic and drastic hypoxic regions within the tumour.

In Fig. 5, a comparison is made between the physical dose profiles when taking into account the effect of OER and when this effect is disregarded. The physical dose profile without consideration to the effect of OER is, technically, the sum total of physical doses related to the 24 ion beams with different energies, which enter the tumour under oxic conditions. In accordance with the definition of OER, Eq. (1) is used to obtain the dose required to produce the same biological effect under similar conditions with the exception of hypoxia being present in the tissue. Therefore, the dose necessary to produce an identical effect, as compared with oxic conditions, is obtained by multiplying the value of the physical dose in oxic conditions at any given point within the tumour by the OER at that same point.

The biological dose is obtained at any given point within the phantom by multiplying the physical dose by RBE. To calculate the

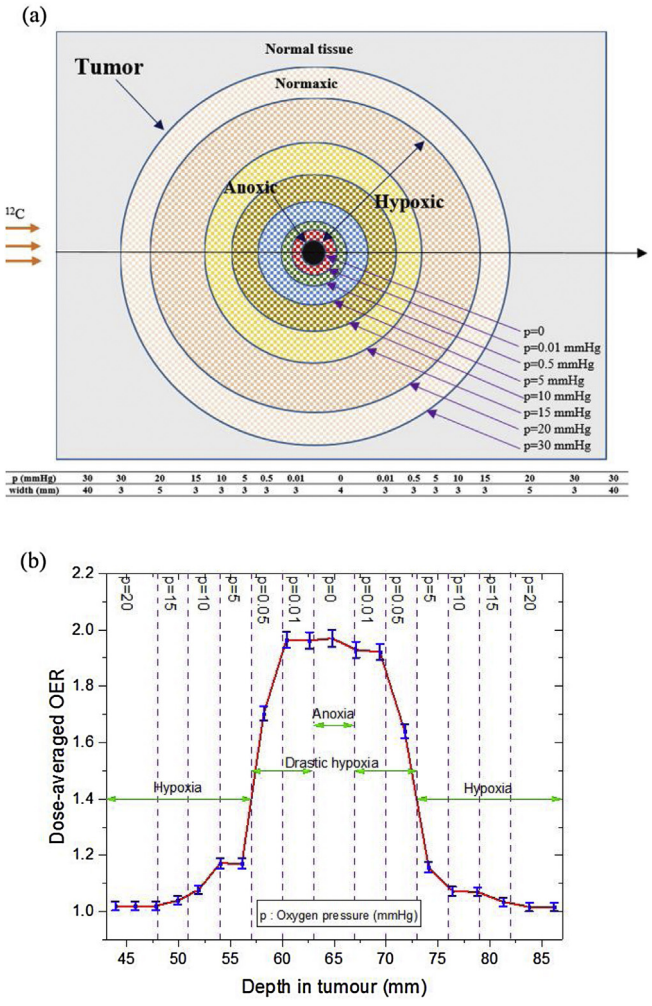

Fig. 4. (a) Cross-section of different parts of a hypoxic spherical tumour and healthy tissue. Different tumour areas are marked with different oxygen pressures. The thickness considered in the calculations for each layer along the ion radiation axis is shown below the figure. (b) The dose-averaged OER in this proposed hypoxic tumour.

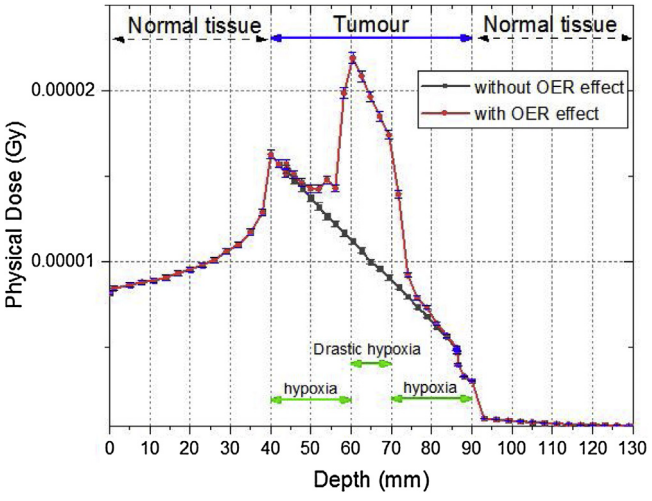

Fig. 5. Physical dose profiles necessary to create a specific biological effect for carbon-ion beams, taking into account the effect of OER (red curve) and no effect of OER (black curve).

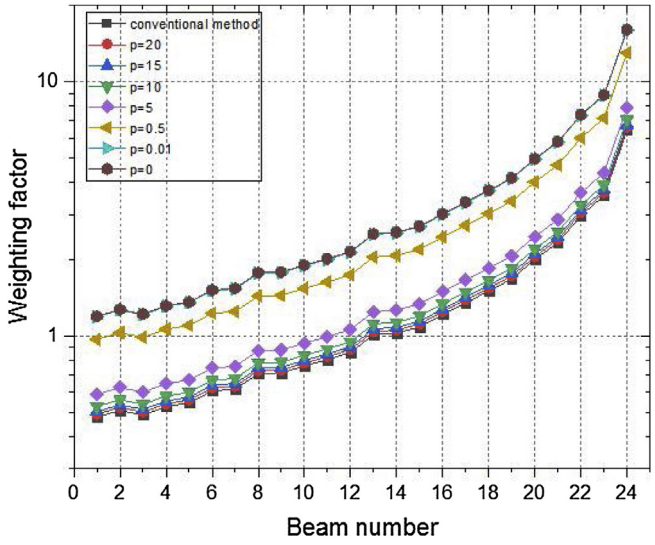

Fig. 6. Weighting factors of intensity in terms of beam number.

RBE, we use Eq. (7), having obtained the values of parameters in the LQ model. For the CHO-K1 cell line, we use the values presented in Table 1. In Tinganelli et al.,[26] the experimental values of α for carbon ion radiation are only available for LET = 100 keV/μm and LET = 150 keV/μm. After testing these values, up to the third decimal place, no discrepancy is observed in the RBE values. Therefore, we select the values of Table 1 as α values for different oxygen conditions with a little bit of approximation. Then, by entering the experimental values of α_{ph} and β_{ph} into Eq. (7) for the CHO-K1 cell line, the RBE values can be calculated. The results of these calculations show that, up to the third decimal place, the RBE can be considered equal to the constant RBE = 4.939 for all points under all three oxygen conditions. By multiplying this RBE value by the physical dose, we will arrive at the biological dose in terms of depth within the phantom.

Now, to calculate the therapeutic gain, we first achieve a desired dose distribution throughout the entire tumour tissue by modulating the intensity of beams with and without taking into account the effect of OER. We then extract the weighting factors for beam intensity through matrix calculations, such that the radiation dose in all parts of the tumour area becomes equal to the maximum dose observed before modulation. The maximum dose that can be delivered to a tumour tissue to cause lethal lesions in the tumour cell while avoiding the overkill effect (i.e. the dose should not be high enough to cause more damage to the cell than is required for cell death) is a biological property. Here, the aim was to modulate the beam intensity to create a uniform biological effect throughout the tumour. If, however, a certain dose value is needed (e.g. twice the maximum dose before modulation) to produce a specific biological effect, the factors will change in the same proportion (e.g. twice). Technically, here, the weighting factors have been calculated for a desired maximum dose throughout the tumour. As previously mentioned, these calculations were carried out based on the method explained in Rezaee.[48] Next, having acquired these weighting factors, we can use Eq. (22) to calculate new weighting factors for each specific oxygen pressure existing throughout the tumour. In Fig. 6, these weighting factors are compared with each other in terms of beam number. Given the logarithmic nature of the vertical axis in this figure, the great differences between the weighting factors at different oxygen pressures are apparent. These weighting factors modulate the intensity of all beams in a way that a homogeneous biological dose is achieved throughout the tumour

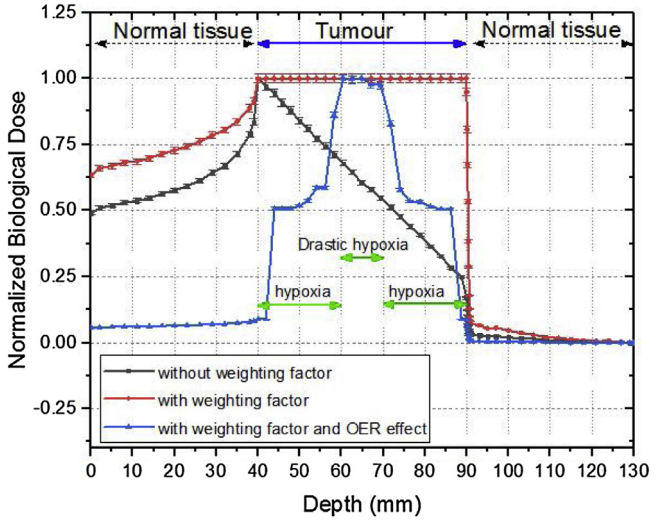

Fig. 7. Comparison of modulated biological doses taking into account the effect of OER (blue curve) and without it (red curve). The total biological dose in the latter case is calculated from Eq. 9 (D_{Tumour}).

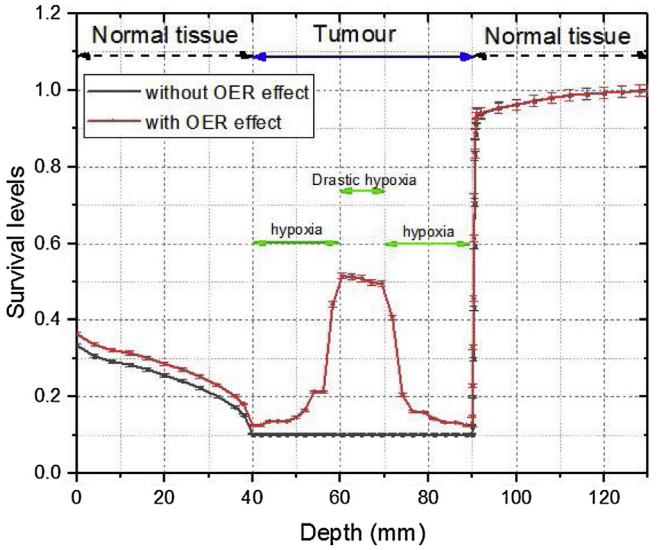

Fig. 8. Comparison of cell survival levels taking into account the effect of OER (red curve), and without it (back curve).

under a certain oxygen condition. This is the purpose of treatment planning in conventional ion therapy.

For a suitable treatment planning that would lead to uniform survival throughout a hypoxic tumour, any part of the tumour with lower oxygen pressure would require a higher dose. Therefore, a proper weighting factor should be determined for any beam peaking at any given hypoxic area using Eq. (22). For the designed tumour in Fig. 4a, beam modulation is carried out as will follow. For this purpose, after multiplying the weighting factors for intensity by biological doses with and without consideration to the effect of OER, we make a comparison between variations of the required dose based on depth, as illustrated in Fig. 7. In this figure, all curves are normalized to unit. The black and red curves represent the non-modulated and conventionally modulated biological dose profiles, respectively, without consideration to the effect of OER. Therefore, according to the conventional treatment planning, the red one should be able to produce the same biological effect across the tumour tissue. The blue curve is derived from applying the effect of OER to the modulated biological dose. Therefore, from this perspective, this dose profile can produce an identical biological effect throughout the tumour tissue. As can be observed, unlike what commonly occurs in conventional treatment planning, when tumour hypoxia levels are taken into account, a significant level of inhomogeneity is required in the radiation doses in order to produce the same biological effect across the entire tumour tissue.

Assuming that a certain biological dose is required to destroy the central tumour cells which are drastically hypoxic, the suggestion of conventional treatment planning would be to deliver that exact dose to all tumour cells. Otherwise, a lower dose of ion radiation will have no destructive effect on those drastically hypoxic cells. On the other hand, in treatment planning based on the effect of OER, this dose will only be delivered to the central regions that contain highly hypoxic cells, and the other areas within the tumour will receive lower doses in accordance with their hypoxia conditions. Therefore, the overall radiation dose will be generally reduced.

To compare the quantitative effects of these two methods, we calculate the average dose delivered to the entire tumour tissue in both methods. The ratio between these two quantities indicates the therapeutic gain. Using numerical dose values normalized to unit, the average doses received by the entire tumour tissue in the conventional method and the novel method equaled $\bar{D}_C = 0.9950$ and $\bar{D}_H = 0.647$, respectively. Therefore, according to Eq. (23), the therapeutic gain is equal to TG = 1.54. In other words, by taking the effect of hypoxia into account during beam intensity modulation in order to create SOBPs in every area, the overall radiation dose can be reduced up to about 1.54 times.

Fig. 7 also indicates a dramatic decrease in the doses delivered to the healthy tissues before the tumour. To assess this result quantitatively, we calculate the ratio of average doses in this area. In the inlet area, the average radiation doses with and without consideration to the effect of hypoxia (corresponding to normalized curves) are equal to 0.369 and 0.603, respectively. Therefore, in this area, the average radiation dose can be reduced by a 1.63 times by taking into account the effect of tumour hypoxia. This can have a positive impact on the protection of healthy tissues around the tumour.

We can also compare the survival curves in these two methods. By introducing the dose value and the values of α and β in Eq. (2), the cell survival rate under a maximum radiation dose of 3.25 Gy can be obtained, as shown in Fig. 8. As can be observed in the figure, disregarding the effect of hypoxia and OER would lead to a survival rate of about 10 % in the tumour cells. Meanwhile, the fact is that by taking hypoxia into account, a much higher survival rate will be achieved compared with the conventional treatment planning. Even in areas with extremely drastic hypoxia, which comprise the central regions of the tumour, the cell survival rate is close to 50 % when hypoxia is taken into consideration. This indicates that treatment planning with no regard to the impact of hypoxia would greatly reduce the actual efficiency of radiotherapy, such that radiation might not even have any therapeutic effects in certain areas within the tumour. This utterly confirms the need to pay attention to the oxygen conditions and oxygen pressure in every region within the tumour in our treatment planning.

The result above is in line with the results of Scifoni et al.[30] In that paper, where the TRiP-OER program was used to calculate the survival rate with and without the OER effect, the survival level in the tumour (which was 10 % disregarding the OER effect) was between 40 % and 50 % in drastically hypoxic regions taking into account an inhomogeneous distribution for the oxygen concentration in the tumour[30] (Section 3–1). Similar results were observed in Ref.[42]

The conventional treatment planning for heavy-ion radiotherapy is based on achieving a homogeneous biological dose distribution throughout the tumour tissue. When trying to achieve a homogenous dose distribution, it is critical to pay attention to the

changes in RBE values in terms of ion energy and depth within the phantom. When we form a spread-out Bragg peak with extremely high homogeneity in the tumour area, it appears that a desirable cell survival rate is achieved. However, the reality of the matter is different in clinical cases.

Achieving a specific biological effect in tumour cells, e.g. cell death, is directly dependent on a factor disregarded in conventional treatment planning. The level of tumour hypoxia, or technically, the oxygen pressure at any given area within the tumour, plays a direct role when trying to induce a specific biological effect. This process has a biochemical cause, which is related to free radical mediation in cellular DNA damage. Therefore, a homogeneous biological dose distribution in conventional treatment planning does not yield satisfactory results with hypoxic tumours.

In this paper, a hypoxic tumour was designed, with variable oxygen pressures and, consequently, variable levels of hypoxia. The energies of carbon ion beams were adjusted in a way that the Bragg peaks would cover the entire tumour tissue. Then, a conventional treatment planning was carried out, in which the weighting factors for beam intensity were calculated in order to form a homogenous SOBP across the tumour tissue. Based on these weighting factors, cell survival rates were also calculated in terms of depth within the phantom. In the next step, the OER was calculated for each area in accordance with its hypoxia level. Subsequently, through a calculation of therapeutic gain, required mean radiation doses were compared between conventional treatment planning and the new method that takes the effect of hypoxia into account. The reduction in the required mean dose in treatment planning with consideration to the impact of OER highlights the positive role of this method. Results from our calculations also reveal that taking the effect of tumour hypoxia into account can result in a significant decrease (about 0.001) in the radiation dose delivered to healthy tissues around the tumour. This can be a good indication of the effectiveness of this type of treatment planning.

Therefore, it can be said that in the case of hypoxic tissues taking into account the effect of OER in the beam intensity modulation, as can be seen in Eq. (22), a compensating dose is deposited that can restore the required survival rate. These results are consistent with studies conducted through the TRiP-OER code, as described in Scifoni et al.[30] This code has the ability to predict the cell survival rate taking into account the effect of OER, as well as compensating the dose and performing inverse planning. Biological effects are also included in OER calculations in this code.[30]

Furthermore, for a tissue composed of several regions with different oxygen pressures, dose compensation can be carried out successfully, but only at the price of significant damages to the healthy tissues around the tumour, which is essential to be prevented. In Scifoni et al.,[30] in order to study dose profiles, a target was designed composed of three regions, namely a normoxic one, a region with a pressure of 0.5, and a region with zero oxygen pressure. In the present article, in order to study more severe gradients in the OER curve and abrupt changes in dose profiles, a target was designed consisting of eight sections with different oxygen pressures, all within the clinical range of hypoxia. Sudden changes in the slope of required doses at the boundaries of every region are evident given the logarithmic nature of the vertical axis in Fig. 5. These changes are made in order to produce an identical biological effect across the target area. This result is consistent with results produced by the TRiP98 code used in Scifoni et al.[30] These sudden changes in radiation dose correspond with the abrupt changes in tissue sensitivity which, in fact, reflect the need to sharply modify the absorption dose required to cross the boundaries of each area.

Another point that needs to be considered is the precise calculation of RBE which depends on the tissue type, radiation energy, and type of irradiating particles.[55] Determining the exact RBE values with minimal error would directly affect the accuracy of required biological doses calculated for each section within the target. The presence of uncertainty in the photon parameters (α_{ph}, β_{ph}) used as primary information in RBE models would create uncertainty in the calculated RBE values.[56] This issue was ignored in the current study, and only approximate RBE values were taken into consideration, a limitation that can be addressed in future research. Obviously, experimental studies under specific oxygen conditions are required to determine the parameters of the LQ model for photon beam radiation. At present, however, there isn't a wide range of experimental data available, which limits the choice of cell lines used in this type of computation. For the purposes of this study, the CHO-K1 cell line was selected, which is one of the few cases with available experimental values for the photon parameters of the LQ model under oxic, hypoxic, and normoxic conditions.[26] Nevertheless, these values are not available for a wide variety of oxygen pressures within the clinical range of hypoxia. Therefore, in order to determine the RBE values with higher accuracy, experimental values are required for photon parameters under a range of different oxygen pressures. This can even play a significant role in the assessment of the correlation between OER and RBE.

5. Conclusions

Calculations for treatment planning were carried out in this study based on partial oxygen pressure and OER, as a function of LET. The main purpose of this article is to modulate the intensity of consecutive beams in the radiation of a hypoxic tumour in order to create a spread-out Bragg peak. In traditional radiation therapy, this modulation is performed in such a way that the same biological dose is delivered to the entire tumour area and, hence, the damage to the entire tumour area is uniformly predicted. The number of lethal lesions in a cell is proportional to the dose delivered to the nucleus of that cell.[43] However, given that radiation resistance is higher for the parts of the tumour that have a low oxygen concentration, a higher dose should be delivered to the cells with a lower oxygen concentration in order to inactivate all tumour cells (whether in the hypoxic region or in other tumour regions). Under these conditions, it can be said that the number of lethal damages caused in all tumour cells (hypoxic and normoxic) would be sufficient to inactivate the cells. Therefore, the intensity of the radiation doses delivered to the tumour should be modulated based on the tumour hypoxia map. This modulation is performed to minimize the damages to healthy tissues surrounding the tumour.

The modulation performed in this paper is analytical and based on the value of OER at each tumour site. When the hypoxia effect is disregarded, the weighting factors for beam intensity are determined based on the dose value in the Bragg peak of each beam. Now, if the hypoxia effect is taken into account, these weighting factors would change and each weighting factor must be multiplied by the dose-average OER value at each point.

To compare the hypoxic tumour responses at different oxygen concentrations, the OER values were calculated according to the depth within the tumour. As expected, OER decreased with increased oxygen content. Therefore, the use of high-LET ions can be taken into consideration for irradiating cells with low oxygen pressure (and, therefore, high OER) in order to optimize the treatment planning.

With the new weighting factors which take into account the hypoxia effect of each point within the tumour, we modulate the radiation to cause uniform damage in a tumour with an inhomogeneous distribution of oxygen concentration. By selecting carbon-ion beams as our high-LET radiation, we calculated the

cell survival rate during the irradiation of this hypoxic tumour with varying oxygen pressures in different regions in an attempt to achieve an optimized treatment planning. We then compared the results of these calculations with outcomes from the conventional treatment planning which does not take the effect of hypoxia into account. Through these comparisons, we found that a major inhomogeneity is required in the dose distribution in order to produce the same biological effect throughout a hypoxic tumour, which is quite different from the common method of treatment. The results also indicated a significant decrease in the mean radiation dose necessary for irradiating a tumour when the impact of hypoxia is taken into account. In this regard, the required radiation dose is reduced by 1.54 times in the tumour tissues and by 1.63 times in the healthy tissues before the tumour. Therefore, paying attention to the hypoxia conditions of a tumour would play a positive and effective role in the optimization of treatment planning in heavy-ion radiotherapy.

Financial disclosure statement

None.

Conflict of interest statement

None declared.

Acknowledgements

None.

References

1. Fyles A, Milosevic M, Hedley D, et al. Tumour hypoxia has independent predictor impact only in patients with node-negative cervix cancer. *J Clin Oncol*. 2002;20:680–687.
2. Hirayama R, Ito A, Tomita M, Tsukada T, Yatagai F, Noguchi M. Contributions of direct and indirect actions in cell killing by High-LET radiations. *Radiat Res*. 2009;171:212–218.
3. Lind BK, Brahme A. The radiation response of heterogeneous tumours. *Phys Med*. 2007;23:91e99.
4. Attix FH. *Introduction to radiological physics and radiation dosimetry*. 1st ed. Weinheim, Germany: WILEY-VCH; 2004.
5. Malinen E, Søvik A. Dose or 'LET' painting – What is optimal in particle therapy of hypoxic tumours? *Acta Oncol (Madr)*. 2015:1–9, http://dx.doi.org/10.3109/0284186X.2015.1062540.
6. Chauvel P. Treatment planning with heavy ions. *Radiat Environ Biophys*. 1994;34:49–53.
7. Sovik A, Malinen E, Skogmo H, Bentzen S, Bruland O, Olsen D. Radiotherapy adapted to spatial and temporal variability in tumour hypoxia. *Int J Radiat Oncol Biol Phys*. 2007;68:1496–1504.
8. Schicker C, von Neubeck C, Kopf U, Kraft-Weyrather W. *Zellkultur bestrahlungskammer*. Europe Patent Specification; 2009. EP090024027.
9. Lyng H, Sundfor K, Rofstad EK. Oxygen tension in human tumours measured with polarographic needle electrodes and its relationship to vascular density, necrosis and hypoxia. *Radiother Oncol*. 1997;44:163–169.
10. Mees G, Dierckx R, Vangestel C, Van de Wiele C. Molecular imaging of hypoxia with radiolabelled agents. *Eur J Nucl Med Mol Imaging*. 2009;36:1674–1686.
11. Padhani AR, Krohn KA, Lewis JS, Alber M. Imaging oxygenation of human tumours. *Eur Radiol*. 2007;17:861–872.
12. Bentzen SM, Gregoire V. Molecular imaging-based dose painting: A novel paradigm for radiation therapy prescription. *Semin Radiat Oncol*. 2011;21:101–110.
13. Krishna MC, Subramanian S, Kuppusamy P, Mitchell JB. Magnetic resonance imaging forin vivo assessment of tissue oxygen concentration. *Semin Radiat Oncol*. 2001;11:58–69.
14. Lin Z, Mechalakos J, Nehmeh S, et al. The influence of changes in tumour hypoxia on dose-painting treatment plans based on 18F-FMISO positron emission tomography. *Int J Radiat Oncol Biol Phys*. 2008;70:1219–1228.
15. Toma-Dasu I, Dasu A, Brahme A. Quantifying tumour hypoxia by PET imaging—A theoretical analysis. *Adv Exp Med Biol*. 2009;645:267–272.
16. Toma-Dasu I, Dasu A, Brahme A. Dose prescription and optimisation based on tumour hypoxia. *Acta Oncol (Madr)*. 2009;48:1181–1192.
17. Sovik A, Malinen E, Bruland OS, Bentzen SM, Olsen DR. Optimization of tumour control probability in hypoxic tumours by radiation dose redistribution: a modelling study. *Phys Med Biol*. 2007;52:499–513.
18. Sovik A, Malinen E, Olsen DR. Strategies for biologic image-guided dose escalation: a review. *Int J Radiat Oncol Biol Phys*. 2009;73:650–658.
19. Thorwarth D, Eschmann SM, Paulsen F, Alber M. Hypoxia dose painting by numbers: a planning study. *Int J Radiat Oncol Biol Phys*. 2007;68:291–300.
20. Thorwarth D, Soukup M, Alber M. Dose painting with IMPT, helical tomotherapy and IMXT: a dosimetric comparison. *Radiother Oncol*. 2008;86:30–34.
21. Thorwarth D, Alber M. Implementation of hypoxia imaging into treatment planning and delivery. *Radiother Oncol*. 2010;97:172–175.
22. Bassler N, Jakel O, Sondergaard CS, Petersen JB. Dose- and LET-painting with particle therapy. *Acta Oncol (Madr)*. 2010;49:1170–1176.
23. Tinganelli W, Durante M, Hirayama R, et al. Kill-painting of hypoxic tumours in charged particle therapy. *Sci Rep*. 2015;5:17016.
24. Furusawa Y, Fukutsu K, Aoki M, et al. Inactivation of aerobic and hypoxic cells from three different cell lines by accelerated (3)He-, (12)C- and (20)Ne-ion beams. *Radiat Res*. 2000;154(5):485–496.
25. Tinganelli W, Durante M, Kraft-Weyrather W. *Radiobiological measurements for radiation therapy: Extension to other ions*. Darmstadt: GSI: GSI Science Report 2010; 2011.
26. Tinganelli W, Ma NY, Neubeck CV, et al. Influence of acute hypoxia and radiation quality on cell survival. *J Radiat Res*. 2013;54:i23–i30.
27. Durante M, Scifoni E, Tinganelli W, Furusawa Y. *GSI-NIRS International Open Laboratory: Measurements of the oxygen effect*. Darmstadt: GSI): GSI Science Report 2011; 2012.
28. Wenzl T, Wilkens JJ. Modelling of the oxygen enhancement ratio for ion beam radiation therapy. *Phys Med Biol*. 2011;56:3251–3268.
29. Stewart RD, Yu VK, Georgakilas AG, Koumenis C, Park JH, Carlson DJ. Effects of radiation quality and oxygen on clustered DNA lesions and cell death. *Radiat Res*. 2011;176:587–602.
30. Scifoni E, Tinganelli W, Weyrather WK, Durante M, Maier A, Krämer M. Including oxygen enhancement ratio in ion beam treatment planning: Model implementation and experimental verification. *Phys Med Biol*. 2013;58(11):3871–3895.
31. Antonovic L, Lindblom E, Dasu A, Bassler N, Furusawa Y, Toma-Dasu I. Clinical oxygen enhancement ratio of tumours in carbon ion radiotherapy: The influence of local oxygenation changes. *J Radiat Res*. 2014;55:902–911.
32. Blakely EA, Tobias CA, Yang TC, Smith KC, Lyman JT. Inactivation of human kidney cells by high-energy monoenergetic heavy-ion beams. *Radiat Res*. 1979;80:122–160.
33. Skarsgard LD, Harrison I. Dose dependence of the oxygen enhancement ratio (OER) in radiation inactivation of Chinese hamster V79-171 cells. *Radiat Res*. 1991;127:243–247.
34. Carlson DJ, Stewart RD, Semenenko VA. Effects of oxygen on intrinsic radiation sensitivity: a test of the relationship between aerobic and hypoxic linear-quadratic (LQ) model parameters. *Med Phys*. 2006;33:3105–3115.
35. Wenzl T, Wilkens JJ. Theoretical analysis of the dose dependence of the oxygen enhancement ratio and its relevance for clinical applications. *Radiat Oncol*. 2011;6:171.
36. Freyer JP, Jarrett K, Carpenter S, Raju M. Oxygen enhancement ratio as a function of dose and cell cycle phase for radiation-resistant and sensitive CHO cells. *Radiat Res*. 1991;127:297–307.
37. Sokol O, Scifoni E, Tinganelli W, et al. Oxygen beams for therapy: Advanced biological treatment planning and experimental verification. *Phys Med Biol*. 2017;62:7798.
38. Krämer M, Scholz M. Treatment planning for heavy-ion radiotherapy: calculation and optimization of biologically effective dose. *Phys Med Biol*. 2000;45:3319.
39. Gemmel A, Hasch B, Ellerbrock M, Weyrather WK, Kramer M. Biological dose optimization with multiple ion fields. *Phys Med Biol*. 2008;53:6991–7012.
40. Krämer M, Durante M. Ion beam transport calculations and treatment plans in particle therapy. *Eur Phys J D - At Mol Opt Phys*. 2010;60:195.
41. Krämer M, Scifoni E, Durante M. *Multi-modal treatment planning with TRiP98*. Darmstadt: GSI: GSI Science Report 2011; 2012.
42. Krämer M, Scifoni E, Schmitz F, Sokol O, Durante M. Overview of recent advances in treatment planning for ion beam radiotherapy. *Eur Phys J D - At Mol Opt Phys*. 2014;68:306.
43. Wang W, Li C, Qiu R, Chen Y, Wu Z, Zhang H, Li J. Modelling of cellular survival following radiation-induced DNA double-strand breaks. *Sci Rep*. 2018;8:16202.
44. Verkhovtsev A, Surdutovich E, Solov'yov AV. Multiscale approach predictions for biological outcomes in ion-beam cancer therapy. *Sci Rep*. 2016;6:27654.
45. Agostinelli S, Allison J, Amako K. Geant4—A simulation toolkit. *Nucl Instrum Methods Phys Res A*. 2003;506:250–303.
46. von Sonntag C. *The chemical basis of radiation biology*. London: Taylor and Francis; 1987.
47. Mairani A, Magro G, Dokic I, et al. Data-driven RBE parameterization for helium ion beams. *Phys Med Biol*. 2016;61:888–905.
48. Rezaee L. Design of spread-out bragg peaks in hadron therapy with oxygen ions. *Rep Pract Oncol Radiother*. 2018;23(5):433–441.
49. Cirrone G, Cuttone G, Mazzaglia E. Hadrontherapy: a Geant4-based tool for proton/ion-therapy studies. *Prog Nucl Sci Technol*. 2011;2:207–212.
50. Romano F, Cirrone GAP, Cuttone G, et al. Applications of Monte Carlo methods to special radiotherapeutic techniques Nuovo Cimento Soc. *Ital. Fis. C*. 2011;34:167–173.
51. Romano F, Cirrone GAP, Cuttone G, et al. A Monte Carlo study for the calculation of the average linear energy transfer (LET) distributions for a clinical proton beam line and a radiobiological carbon ion beam line. *Phys Med Biol*. 2014;59:2863–2882.

52. Surdutovich E, Solov'yov AV. Multiscale approach to the physics of radiation damage with ions. *Eur Phys J D - At Mol Opt Phys*. 2014;68:353.
53. Surdutovich E, Solov'yov AV. Calculation of survival probabilities for cells exposed to high ion fluences. *Eur Phys J D - At Mol Opt Phys*. 2018;72:140.
54. Kase Y, Kanematsu N, Kanai T, Matsufuji N. Biological dose calculation with Monte Carlo physics simulation for heavy-ion radiotherapy. *Phys Med Biol*. 2006;51:467–475.
55. Grzanka L. *Modelling beam transport and biological effectiveness develop treatment planning for ion beam radiotherapy*; 2013.
56. Friedrich T, Weyrather W, Elsasser T, Durante M, Scholz M. Accuracy of RBE: Experimental and theoretical considerations. *Radiat Environ Biophys*. 2010;49:335–349.