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The importance of hypoxia & hypofractionation for CyberKnife stereotactic radiosurgery

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Intracranial and extracranial stereotactic radiotherapy using the CyberKnife system permits very precise treatments with a minimum amount of normal tissues in the treated area. Hypofractionation is becoming an increasing issue in these treatments. By giving a high dose per fraction, it could be possible to increase the therapeutic ratio for some tumours with a low α/β ratio, as these are sensitive to a high dose fraction or dose rate. This can counteract some of the hypoxia induced radioresistance of the tumour.

Znaczenie niedotlenienia oraz zmniejszenia liczby dawek promieniowania w radioterapii stereotaktycznej z zastosowaniem systemu CyberKnife

Wewnątrz i zewnątrzczaszkowa radioterapia sterotaktyczna z zastosowaniem systemu CyberKnife umożliwia precyzyjne działania z minimalnym uszkodzeniem zdrowych tkanek. Zmniejszanie liczby dawek staje się coraz istotniejszym problemem – podając dużą dawkę na frakcję możliwe jest zwiększenie odsetka odpowiedzi, szczególnie ze strony guzów o niskim stosunku α/β, ponieważ są one wrażliwe na wysokie dawki we frakcji lub czas podania dawki. Może to zapobiegać spowodowanej hipoksją oporności na radioterapię.

Key word: stereotactic radiotherapy, intracranial tumours
Słowa kluczowe: radioterapia stereotaktyczna, guzy śródczaszkowe

Introduction

In a recent publication [1] Adler et al defined radiosurgery as 'precise targeting of cross-fired radiation beams to abnormal tissues with an abrupt fall-off of radiation doses to surrounding normal tissues' and as 'a procedure that involves the active participation of a surgeon and in which spatially accurate and highly conformal doses of radiation are targeted at well-defined structures with an ablative intent'. This demonstrates that it is of critical importance to properly define new treatment strategies and techniques [2].

Stereotactic radiosurgery is a rapidly emerging fied within radiation oncology which is expanding from the initial limitation of only cranial radiotherapy to encompass extracranial radiotherapy applications. The early restrictions to its use for was due to factors such as the requirement for a non-mobile target, and a lack of very precise stereotactic imaging software: e.g. for CT, MRI and angiography. It was necessary to use only a limited number of fractions in order to avoid difficult repeat set-ups which were often invasive. Also, due to the radiobiology of tumours and normal tissues the volume and the dose given in a single shot had to be limited to avoid extensive necrosis of the irradiated surrounding normal tissues [3, 4].

Expanding applications from cranial to extracranial malignant and non-malignant conditions presents requires a solution to the problem of how to deal with mobile organs and what restrictions are necessary. The new developments of imaging technology such as in SPECT, MRI and PET, provides more useful information for the definition of the target volume and surrounding normal tissues. The ultimate tool would be to track the tumour in real time.

The limitation for treatment planning and delivery is the complexity of the precision process. With the CyberKnife being a frameless system, unlike the Gamma Knife where a head frame is essential, this opens up a wide range of current and future possibilites for extracranial stereotactic radiosurgery. Although it must be realised that the more complex the overall systems the longer it will take to define the target, set up the patient and deliver treatment. This implies that there is no alternative other than decreasing the total number of fractions [5].

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Changing from a single shot with a low dose to a limited volume in order to obtain necrosis of normal tissues (e.g. arteriovenous malformations) to multiple shots (fractionation) with a curative total dose to a large volume (i.e. tumours and their margins which form the CTV) to obtain tumour cure and a protection of surrounding normal tissues will need extremely precise techniques and machines as well as a compromise on the number of fractions to be delivered [5]. This compromise must not be compared to that chosen for medico-economical reasons. In other word, decrease the total number of fractions delivered in order to potentially increase the number of treated patients and hence improve the cost implications.

Extracranial stereotactic radiosurgery

To properly extend stereotactic radiotherapy from cranial to extracranial sites requires extremely precise tools and a frameless system such as that of the CyberKnife. Practice guidelines for stereotactic body radiation therapy were published in 2004 by the American Society for Therapeutic Radiology and Oncology and the American College of Radiology [6].

The conclusion by ASTRO and ACR was as follows. 'The quality of the stereotactic body radiation therapy [SBRT] program is only as good as it's weakest link. High spatial accuracies are expected and time constraints are relatively short. Since SBRT uses either single fraction treatment or a hypofractionated regimen, there is little chance for adjustment once treatment has been initiated. This demands considerable time for planning and treatment verification by the radiation oncologist and medical physicist'.

Hypofractionated regimens have been widely used in palliative settings such as for treatment of bone metastases in order to rapidly relieve pain by quickly obtaining anti-tumour and anti-oedema effects. In some metastatic diseases such as those of liver and lung, a long-term tumour response can be obtained by delivering a high biological effective dose (BED) in the short-term. This limits the constraints for the patients, even if their mean survival remains short.

Hypofractionated regimens can only be considered as a sole curative treatment if precise definition and tracking of the target is obtained [7]. Furthermore, a biological rationale must exist in order to consider implementing the technique in patients.

For prostate cancer, there is currently a debate on hypofractionated treatment [8]. The target is well defined using MRI, the α/β ratio may be low in most of the tumours and the tracking can be obtained. However, the rectal mucosa is very close to the surface of the prostate, < 5 mm, and some morbidity can be expected. For prostate cancer, CyberKnife treatment has been simulated with inverse planning, using non-coplanar non-isocentric arcs and compared to IMRT delivered with a linear accelerator [9]. Conformal isodose curves and dose-volume histograms have been drawn with superior DVHs obtained with the Cyberknife with regard to sparing of normal tissues. Preliminary clinical experiences have also been published and for spinal lesions, a regimen of 11-25 Gy in 1-5 fractions has been used with good results at six months follow-up [10].

Finally, it is noted that a single high dose can be delivered as a boost before or after fractionated treatment. This has been performed in intraoperative radiotherapy and as a boost for brain and for some gynaecological tumours. Currently, we are now close to what is achieved by a HDR brachytherapy boost after external beam treatment.

For brain tumour metastases, the recently published RTOG 95087 study demonstrated that there was a benefit in terms of survival for patients with a single unresectable metastasis, when delivering a stereotactic radiosurgery boost after whole brain irradiation: from 24 Gy in 2 cm diameter to 15 Gy in 4 cm diameter lesions [11], Tables I and II. However the risk of tissue necrosis remains high and limits the hypofractionation used and the normal tissue volumes irradiated [7]

Table I. Causes of death from the RTOG 9508 study [11]. WBRT = whole brain radiation therapy

Cause of death	WBRT & stereotactic	WBRT alone
	N = 137	N = 149
Brain metastases	39 (28%)	46 (31%)
Cancer: other site	69 (50%)	80 (54%)
Radiotherapy complications	1 (1%)	0 (0%)
Unknown	12 (9%)	8 (5%)

Table II. Multivariate survival analysis for the RTOG 9508 study [11]. RPA classes = recursive partitioning analysis classes for brain metastases

	Number of cases	Mean survival time (months)	P-value	Association with a better prognosis
The overall study	327			
RPA class 1 *versus* class 2		2.254	< 0.0001	Class 1
Histology		1.348	0.0121	Lung primary
WBRT *versus* WBRT + stereotactic		not known	0.1249	Not significant
Single metastasis	184			
RPA class 1 *versus* class 2		2.897	< 0.0001	Class 1
WBRT *versus* WBRT + stereotactic		not known	0.0533	Not significant

Hypoxia and radiation therapy

The biological effectiveness of low LET ionising radiation is mostly due to the oxygen present at the time of treatment in the cell environment [12, 13]. Oxygen availability depends on oxygen supply and tissue oxygenation will result directly from O_2 availability and from the respiration rate of the cells. In tumours, tissue vascularisation is qualitatively poor with shunts, collapsed vessels and high interstitial pressure [14, 15]. Hypoxic cells are present in rodent and xenografted human tumours and it has been known for a long time that the absence of oxygen in tumours is a factor of resistance against ionising radiation [16-18]. The low tumour oxygen tension could also be a factor of resistance for treatment with some cytotoxic drugs [19].

Physiology of hypoxia

In mammalian cells, most of the energy for cellular life arises from oxidation and reduction reactions with ATP synthesis [20]. Large variations in the density of vessels have been demonstrated during tumour growth and hypoxia is present not only in large tumours with necrosis, but also in small tumours [21, 22]. This is related in part to the growth site, tumour cell density and to variations in vascularisation [23]. The oxygen supply for a defined tumour at a precise time and at a specific point will depend on the tumour blood flow at that time. Cell killing by ionising radiation occurs through DNA damage. For low LET radiations the most frequent targets are water molecules, which become converted to free radicals leading to DNA damage.

The oxygen enhancement ratio (OER) under oxygenation conditions versus anoxic conditions has a range of 2.5-3.5 for X-rays, gamma rays and electrons [13]. However, for heavy ions and neutrons, the radiation effects are much less dependent on free radicals and have a much lower OER which is close to 1.0. Although the effects of hypoxia on radioresistance have been well characterised, there are other tumour characteristics which have an influence on the curability of tumours.

For example there is the biological response to hypoxia and at the genetic transcription level, several genes have been shown to be regulated by hypoxic stimuli [24, 25]. A hypoxia inducible transcription factor (HIF-1) which was initially identified as the specific transcription factor involved in the control of the erythropoietin gene, is now known to regulate multiple cellular processes such as the expression of vascular endothelial growth factor (VEGF) [26, 27]. In addition to the HIF-1 response which is specific to hypoxia, other less specific responses also occur as a result of hypoxic stress including p53, AP-1, NF-kB, SP-1 and the proto-oncogenes Jun and Fos. These diverse effects on gene transcription are consistent with the ubiquitous and fundamental effects of hypoxia on cellular metabolism [28-32].

Studies of oxygen partial pressure in tumours

Hypoxia is present to a significant degree in solid tumours [20]. The blood vessels do not carry blood as efficiently as normal vessels and are not under regulatory control and tumours typically contain normoxic, hypoxic and necrotic regions [23]. In 1956 Thomlinson and Gray constructed their model of tumour oxygenation with cords of oxygenated cells surrounding each capillary [22]. Tissues can maintain full oxygenation if they are within 100 μm of a perfused capillary. However, at distances between 100 μm and 150 μm, the tissues are chronically hypoxic. Beyond 150 μm the tissues are anoxic and necrotic.

It has since become apparent that the situation in tumours is far more complex due to the unorganised nature of tumour vasculature. Most of the oxygen in normal tissues is distributed via arterioles and tumour capillaries are inadequate since blood is carried by these vessels. Changes in the perfusion and/or oxygenation of many tumour cells is not only dependent on location within the tumour but varies over time by as much as 10-fold [32, 33].

Angiogenesis and loss of cell adhesion

Angiogenesis is the formation of new blood vessels. However, hypoxia is also an important phenomenon in tumour development. If a tumour cannot induce the formation of new vascular connections with the adjacent blood vessels, it is unable to grow beyond the size limits imposed by oxygen diffusion: a diameter of < 1 mm). For this reason, an angiogenic phenotype appears to be essential for development of a malignant tumour [27, 32]. A principal positive regulator of angiogenesis in normal tissues is VEGF which is under control of HIF-1. Also, angiogenesis is highly responsive to hypoxia in normal tissues or in tumours [34]. The resulting vascularisation not only promotes growth but also increases the potential for metastatic spread because of improved access of malignant cells to the bloodstream.

Genetic instability and selection of malignant phenotype

Hypoxia itself may promote increased genetic instability [35]. Experimental studies have shown an increase in metastatic potential linked specifically with reoxygenation following hypoxia. This reoxygenation stage induces cell cycle perturbations and DNA overreplication. The selection pressure exerted by hypoxia may also favour other characteristics that produce a more malignant phenotype [36]. One example is loss of functional p53 which is the most frequently mutated gene in tumours.

It suggests the hypothesis that its function is to protect the organism by preventing the replication of cells with damaged or mutant DNA. In normal cells, hypoxia can induce p53 mediated apoptosis. In addition, a hypoxic environment applies a selection pressure favouring, in

any genetically unstable tumour lineage, the survival of cells with mutant p53. This selection has been demonstrated in oncogenically transformed tissue culture cells [30].

Techniques of oxygen tension measurements

Methods for measuring the oxygen present in tissues can be divided into invasive and non-invasive techniques. The ideal method for measurement would need to be accurate and reproducible, to have a good spatial resolution, a sensitivity in the clinically relevant range and to be user friendly and safe. For clinical usefulness, a non-invasive method should be preferred but most of the available techniques give only an indirect estimation of oxygen pressure in the tissue assessed [37].

For the polarographic technique, an electrical current is generated between a cathode (probe) and an anode. The variations in oxygen concentration in the tissue will induce oxidation of the electrode, leading to modifications in the current which is proportional to pO_2. The electrode must have a minimal oxygen consumption, should avoid tissue compression, have a fast response, be easily calibrated with minimal drift and specific of oxygen. The exact volume of oxygen detection in tissues by modern probes corresponds to the micro-environment of the tumour cells.

The principle of hypoxia markers is based on the fact that the binding of hypoxic cells radiosensitisers, as for example 2-nitroimidazole, to tumour cells is dependent on oxygen concentration [38, 39]. The limitation of these techniques is due to the uncertainty in the bio-distribution of the marker due to variations in tumour blood flow and on the exact correlation between the intensity of the binding and the partial oxygen pressure in the tissue. This means that the measure will serve more on an individual monitoring of oxygen distribution in time, than on a comparison between different tumours.

The binding can be detected by invasive techniques such as biopsy or immunochemical; or by non-invasive techniques such as PET or MRI. Hypoxia is detected at a cellular level with the invasive techniques (biopsy or needle) and can be associated with the evaluation of other parameters (proliferation). For non-invasive techniques, the micro-environment is by definition not modified by the sampling, but the recording corresponds more to an evaluation of tissues than of cells.

In a National Institutes of Health consensus workshop held in November 1992, the various advantages and disadvantages of such techniques were reviewed [40]. For measuring tissue oxygenation it was concluded that a comparison of techniques would be advisable with oxygen electrodes serving as the 'gold standard'. The different techniques used for measuring oxygen tension in tumours might give different information than information on 'biologically significant' tumour hypoxia.

pO_2 distribution in tumours

Oxygen tension has been measured in head and neck, cervix, brain tumours, melanoma and sarcomas [41-47]. In metastatic neck nodes from a primary squamous cell carcinoma of the head and neck (cancer of the oro-pharynx in 31/35 patients) pO_2 measurements were performed immediately before treatment (surgery or radiotherapy) [41]. The median pO_2 was 9 mmHg with a mean pO_2 of 21 mmHg. Low pO_2 values of <10 mmHg were found in 83 % of the tumours. The tumour-to-tumour proportion of very low values of <2 mmHg varied widely in the range 2.5-100%.

Tumour pO_2 values generally varied along the electrode tracks but in general, there appeared to be no systematic variation in pO_2 as a function of the electrode position in the tumour. That is, no decrease in pO_2 from the superficial to the deep part of the tumour. The pO_2 distribution did not differ according to the age of the patient or to the histological grade of the tumour.

The distribution of oxygen tension varied greatly from one tumour to another, indicating differences in tumour oxygenation between patients with the same histological tumours: inter-tumor variability. Recently, pO_2 measurements have been performed in prostate cancer. The median pO_2 was in the range 0.2-57.3 mmHg with a median of 4.5 mmHg. A large intra-tumour variability was found [42].

Factors influencing pO_2 variation

The factors studied which can possibly influence pO_2 variation are (1) tumour size, (2) haemoglobin concentration, (3) tumour treatment and (4) carbogen breathing.

With regard to (1) tumour size in head and neck tumours, a trend has been demonstrated showing a decrease in the median pO_2 (there is an increase in the frequency of very low values < 2 mmHg) associated with an increase in tumour size: N2 versus N3, $p = 0.012$ [41]. The same trend was also found in sarcomas [44]. With regard to (2) haemoglobin concentration, no correlation between haemoglobin level and intratumour oxygen tension distribution was found in all studies except for one.

With regard to (3) tumour treatment the variations in tumour oxygenation during very accelerated radiation therapy for advanced head and neck carcinoma have been studied. For normal tissues outside the radiation field, the oxygen distribution was not significantly increased during treatment. There was a pooled median pO_2 of 38 mmHg before treatment which was 46 mmHg after two weeks of treatment. For tumours, the pooled median pO_2 was 13 mmHg before treatment and 33 mmHg after two weeks of treatment. This difference was significant, $P=0.05$. Very low values of <2 mmHg represented 20% of the recorded values before treatment and 10% after two weeks. Low pO_2 values of <10 mmHg accounted for respectively 45% and 25% of the recorded values before

and after treatment [43]. With regard to (4) carbogen breathing an efficient method to decrease the percentage of low pO^2 values in tumours is to breathe carbogen, or pure oxygen, throughout every radiotherapy treatment fraction [45]. Oxygen tension has been recorded in tumours before and at the time of carbogen breathing. A decrease in the percentage of very low values of < 2 mmHg was obtained in 9/13 patients.

Predictive value of pO_2 measurements

The predictive value of pO_2 measurements for treatment response has been demonstrated in cervix and head and neck tumours and in sarcomas. In 35 patients locally advanced head and neck tumours, Nordsmark et al demonstrated that pO_2 was a significant and independent parameter for predicting local tumour control after treatment exclusively by radiotherapy [46]. Actuarial tumour control probability at two years was 70% for well oxygenated tumours, versus 36% for poorly oxygenated ones, P=0.01. The parameter studied was the fraction of pO_2 values <2.5 mmHg. In an investigation of patients with soft tissue sarcoma, well oxygenated tumours (median pO_2 >10 mm Hg) were associated with a two-fold higher probability of disease free survival at 18 months than were those with poorly oxygenated tumours (median pO_2 <10 mm Hg), P<0.01 [47]. It was found that treatment failure and relapse were due primarily to distant metastases. It was concluded that tumour hypoxia was a powerful prognostic factor for metastatic potential.

Finally, it can be said that most of the solid tumours in man contain areas of hypoxia. The measurement of tumour pO_2 can be considered as a predictive indicator of treatment response in cervix and head and neck tumours. Oxygen tension can be modulated by carbogen breathing and can vary during radiation therapy [48, 49]. Also, even if tumour hypoxia is a highly relevant parameter for treatment outcome, the best parameter to demonstrate predictive tumour hypoxia is still not clearly defined.

Does hypofractionation counteract hypoxia?

Role of fractionation

Repair of sublethal damage, tumour repopulation and the level of tumour (re-) oxygenation are the main factors which determine the outcome of any particular irradiation schedule. Cell cycle redistribution is less important. The only problem is to understand the range of dose and dose rates at which the variation in biological effectiveness is of clinical significance. This depends on two main factors, repair capacity and repair kinetics, which will change from tissue to tissue.

The analysis of radiation effects in vivo reveals important differences between fast and slow proliferating tissues: that is, early and late effects [3, 4]. These two groups of tissues of course differ in their latency before reacting to an irradiation, but they also differ in their sensitivity to alterations in the fraction size in a fractionated irradiation schedule. Variations in the dose per fraction have an impact on tolerance to radiation which is comparatively greater in late than in early reacting tissues or tumours. The skin for instance, which is a typical acute reacting tissue, is relatively insensitive to decreasing the dose per fraction beyond about 2-3 Gy. This difference in fractionation sensitivity is interpreted as reflecting differences in sublethal damage repair capacity and constitutes the basis of the differential effect of fractionation on early and late responding tissues.

In changing the fractionation, one would have considered calculating an isoeffect dose. However, it must be stated that the α/β linear-quadratic (LQ) model is only valid for fractionated treatment in the range 2-20 Gy per fraction with uncertainties on α/β and on $T_{1/2}$ Most of the calculations are made with the LQ model assuming mono-exponential repair kinetics rather than multi-exponential.

In summary, any increase in dose per fraction (or in dose rate) will have a greater biological impact on slow responding normal tissues than on tumours. To expect any increase in therapeutic ratio, the amount of normal tissues in the treated volume will have to be as reduced by as much as possible. This is what can be achieved using CyberKnife stereotactic radiosurgery, unlike the use of conventional fractionated radiation therapy.

Hypofractionation in clinical practice

Using large doses per fraction for curative tratment is still considered to be a challenge. A recent paper by Fowler et al studied the comparison of a 2 Gy equivalent dose with large fraction doses [3]. A new concept was developed: the prescription isodose volume PIV, for comparison with the planning treatment volume PTV in models of large fraction doses in lung carcinoma. A regimen of 35 x 2 Gy was compared to 3 x 15 Gy or 4 x 12 Gy (>110 $Gy_{\alpha/\beta 10)}$ used in stereotactic hypofractionated schemes.

If in a tumour with 10^{10} resistant cells reoxygention is incomplete with 1% remaining hypoxic cells, then 8 log cells will remain hypoxic and the total biological dose will have to be at least three times higher to control the tumour. If OER is only 2.5 and not 3, then fractions of 3 x 23 Gy will be necessary and not 3 x 15 Gy. However, this must be placed in perspective with the evaluated number of patients having hypoxic tumours. This is an evaluation which is still difficult clinically. Furthermore, hypoxia remains only one of the potential factors affecting radioresistance. Also, the number of critical factors (such as repopulation, cell kinetics, proliferation and oxygenation) may vary during the course of a fractionated radiotherapy regimen [50].

Finally these biological concepts must be seen in the perspective of treated volumes. If the minimum PIV is considered, a PTV of 5 cm in diameter will be decreased to 4 cm in diameter by a reduction in the margin of 0.5 cm (i.e. a 20% decrease) but the volume will be halved (65.5 cm^3 to 33.5 cm^3).

Conclusions

It is logical to consider increasing the dose per fraction in stereotactic radiosurgery where the target can be precisely located and the irradiated volume can be limited. However, only long-term clinical evaluation will allow a generalisation of the concept in curative treatment for the most commonly treated human tumours. Fully integrated robotic radiosurgery systems such as the CyberKnife may allow us to achieve this goal [51].

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