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Calculation of the Inactivation Cross Section of V79 Cells by Protons in Radiotherapy

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حساب المقطع العرضي لتخميل خلايا V79 بواسطة البروتونات

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الملخص: الهدف: من أجل الاستخدام الكفوء للبروتونات في العلاج بالأشعة ينبغي أن تتوفر المعرفة التفصيلية عن الآلية المطلوبة للطريقة البيولوجية – الإشعاعية ذات العلاقة. يعتبر المقطع العرضي للتعطيل وسيلة مفيدة لكشف وسيلة التفاعل . ولهذا تم حساب المقطع العرضي للتعطيل لأشعاع البروتون على خلايا V79 بواسطة معطيات البقاء المنشورة . **الطريقة:** استخدمت المعادلة الخطية التربيعية لمطابقة منحنيات البقاء بينما استعملت مُتَتَابِعَة الف لحساب المقطع العرضي. ورسم المقطع العرضي (σ) كدالة لمتوسط المسار الحر (λ) وانتقال الطاقة الخطي. **النتائج:** المقطع العرضي σ مقابل منحنى λ يظهر منطقة إشباع بين $\lambda \geq 2$ نانوميترو $\lambda \leq 5$ نانوميتر. في هذه المنطقة قيمة المقطع العرضي للتخميل تكون تقريبا مساوية للمساحة الهندسية لقطعة الحَمْضُ الرِّيْبِيُّ النَّوَوِي المَنْزُوع الأوكسِجين (حوالي 4 μm2). كما لوحظ هذا الإشباع أيضا في LET -σ - منحنى انتقال الطاقة الخطي. **الخلاصة:** هذا يدل على أن قطعة من الحَمْضُ الرِّيْبِيُّ النَّوَوِي المَنْزُوع الأوكسِجين تكون في خطر عند عبور بروتون واحد.

مفتاح الكلمات: المقطع العرضي للتخميل . ضرر الحَمْضُ الرِّيْبِيُّ النَّوَوِي المَنْزُوع الأوكسِجين . متوسط المسار الحر . التأين . نوعي أولي . انتقال الطاقة . خطي.

Objective: For efficient applications of protons in radiotherapy, detailed knowledge of the corresponding radiobiological mechanism is necessary. The inactivation cross section is a useful tool to explore the interaction mechanism. Hence, the inactivation cross section for proton irradiation on V79 cells has been calculated using published survival data. ***Method:*** The linear quadratic equation was used to fit the survival curves and the parameter α was used to calculate the cross section. The cross section σ is plotted as a function of the mean free path λ and the linear energy transfer LET. ***Results:*** The cross section σ-versus-λ curve shows a saturation region between λ≥2 nm and λ≤5 nm. In this region, the inactivation cross section value is about the same as the geometrical area of the DNA segment (≈4 μm²). Such a saturation is also seen in the σ-LET curve. ***Conclusion:*** This implies that one DNA segment is at a risk upon the traversal of a single proton.

Key words: Radiotherapy; Inactivation cross section; DNA damage; Mean free path; Ionisation, specific primary; Energy transfer, linear.

Advances in Knowledge

Cell inactivation by ionising radiation has been studied in many ways such as survival curves shapes, dose rate survival dependence, and inactivation cross section etc. Watt and his co-workers were the first to adopt the cross section trends in an attempt to find a universal manner of radiation action regardless of target and radiation type.[1-4] They analysed published data for inactivation of enzymes, viruses and mammalian cells by accelerated ions and electrons, their analyses were based on the relationship between the inactivation cross section σ and the specific primary ionisation I, or the mean free path λ; in order to compare various kinds of cells; the inactivation cross section was normalized to the geometrical cross section. They observed discontinuities in the σ-I and σ-λ curves for double stranded mammalian cells, which were not seen in single stranded cells such as viruses and enzymes. The

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conclusion was that the dominant mechanism for reproductive death is determined by the matching of the mean free path between ionisations to the strand separation in double stranded DNA. Successive work was done on V79 cells inactivated by accelerated ions,[5] the same conclusion was drawn in charged ions inactivation; however, the electrons showed a cross section approaching an order of magnitude smaller than that for heavy ions at the same λ. This work is concerned with the inactivation cross section of V79 cells by protons and its relation to the mean free path λ and the linear energy transfer (LET).

Application to Patient Care

Systematic investigation of the biological effects caused by different radiation qualities are powerful tools for obtaining a better insight into the mechanism of radiation action in biological matter. There is now a substantial amount of theoretical work that addresses the relationship between radiation quality and the amount of energy deposited in the DNA helix and the subsequent production of DNA. It is commonly assumed that different particles having the same LET have similar biological effectiveness. However, experimental data gathered in recent years provided evidence for Z dependence of the biological effectiveness of radiation.[6] Moreover, experimental studies on the biological effects of low energy charged particles in V79 cells has shown that low energy (high LET) protons are more effective in cell inactivation than other heavy charged particles as alpha particles. As accelerated protons have already proved their usefulness in clinical radiotherapy, the detailed understanding of the underlying radiobiological mechanism is necessary in order to take the advantage of their possible benefits and to optimize treatment procedures in individual cases.

The increasing importance of protons in radiotherapy makes it essential to understand the damage mechanism of these particles. The interaction cross section has proved to be a good physical quantity to study the size of the effect and to give some idea about the physical mechanism in various fields of physics. Theoretical investigations by physicists, radiobiologists and physicians have pointed out the superiority of charged particles in radiotherapy treatment. Among these, protons have been found to provide the most clinical advantages for many reasons such as their ability to concentrate the dose in more discrete target volumes and the low scattering compared to electrons. Also protons are considered to be a low-LET radiation.[7] This makes it essential to know the main physical processes involved in the interaction of proton beams with living cells, which are a manifestation of radiation quality and from which physical parameters descriptive of the quality may be extracted and used for other radiation types.

Whilst the biological processes initiated by the radiation action are thought to be immensely complex, there seems to be a general consensus that double strand breaks induced in the cellular DNA constitute the predominantly important lesions;[2, 8] however, there are still considerable doubts about the most appropriate physical parameters to use for specification of radiation quality. Empirical considerations suggest that energy deposition parameters such as LET may be inappropriate.[3] An approach predicted by Watt and his co-workers suggests that the mean free path for primary ionisation plays a major role in the DNA damage.[3, 5] They found that a structure is observed at a mean free path for ionisation of between 1.5 and 2.0 nm and occurs only in those targets containing double stranded DNA. This was explained as a matching between the DNA strand separation (1.8-2.0 nm) in the double stranded DNA and the mean free path for primary ionisation. In other words, maximum damage occurs when the mean free path for primary ionisation is of the same order of magnitude as the DNA strand separation.

As part of an investigation into a better method for specifying radiation quality for application to damage modelling, we have surveyed published survival curves data for proton irradiation on Chinese hamster cells V79. The effective cross section was extracted from these data after fitting the survival curves to the linear quadratic equation; the initial slope α from the fitted equation was used to determine the cross section as this avoids any complications with cell recovery. Cross section relation to the mean free path λ and the linear energy transfer LET are given. Our results show evidence supporting the predictions that the double strand breaks in the DNA are the main cause of damage to mammalian cells.

Table 1: ***Energy dependence of fitting parameters and cross section in the inactivation of V79 cells by protons***

Energy (MeV)	LET (KeV/μm)	λ (nm)	α $(Gy)^{-1}$	β $(Gy)^{-2}$	σ $(μm)^2$	Adjusted R=square	References
0.04	72.3	1.13	0.171	0	1.98	0.9921	Belli[12]
0.26	58.9	2.1	0.367	0	3.4586	0.9987	Belli[12]
0.57	37.8	4.32	0.5952	0.0022	3.5998	0.9972	Belli[13]
0.64	34.6	4.85	0.6157	0.0215	3.4085	0.997	Belli[13]
0.73	34.5	5.3947	0.625	-0.065	3.45	0.9992	Belli[9]
0.76	31.9	5.6579	0.6538	0.0945	3.337	0.9976	Folkard[11]
0.84	30.4	6.0088	0.6938	-0.046	3.3746	0.9952	Belli[9]
1	26.5	7.3246	0.733	0	3.1	0.993	Schettino [14]
1.15	24.7	8.3772	0.6894	0.0809	2.7245	0.9987	Folkard[11]
1.16	23.9	8.5526	0.6904	0.1363	2.7166	0.999	Belli[9]
1.41	20	10.417	0.649	0.0441	2.0768	0.9994	Belli[13]
1.7	17.8	12.588	0.5903	0.0493	1.6812	0.9973	Belli[9]
1.9	16.72	14.079	0.3207	0.1061	0.8579	0.9968	Folkard[11]
3.2	10.99	22.939	0.24	0.046	0.4219	1	Schettino [14]
3.36	11.111	24.167	0.1851	0.1535	0.329	0.9992	Belli[9]

METHOD

Survival curves data for proton irradiation of V79 cells were taken from literature, scanned and fed to the computer as image files readable by MATLAB (MATrix LABoratory - a numerical computation programme) and transferred into figure files in pixel units. Calibration curves relating the pixel units and the real coordinates units were used to extract the real data points. Hence, a complete set of survival curves data points was obtained; the error bars were not taken into consideration. This was done for 15 survival curves ranging in energy between 0.04-3.6 MeV.

CROSS SECTION CALCULATION

The survival curves obtained were fitted to the linear quadratic equation [8, 9] using the MATLAB fitting tools:

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

Where S is the surviving fraction, D is the dose in Gy and α and β represents the contribution to cell destruction. The goodness of fit was measured by the adjusted R-square value, which takes into account the number of degrees of freedom in the fitted equation (two in this case). R-square is defined as the ratio of the sum of squares of regression and the total sum of squares about the mean; it can take any value between 0 and 1, with a value closer to 1 indicating a better fit. R-square values obtained from the fit were very close to 1. Table 1 shows the proton energies used, the values of α and β obtained from the fit and the adjusted R-square value for each fit. The α-values which represent the initial slope of the survival curve were used to calculate the inactivation cross section which is defined by the equation. [4, 5, 10]

$$\sigma(\mu m^2) = \frac{0.16 * L_T(KeV/\mu m) * \alpha(Gy)^{-1}}{\rho(g/cm)^3}$$

L_T is the track average linear energy transfer and ρ is the tissue density taken as equal to 1.

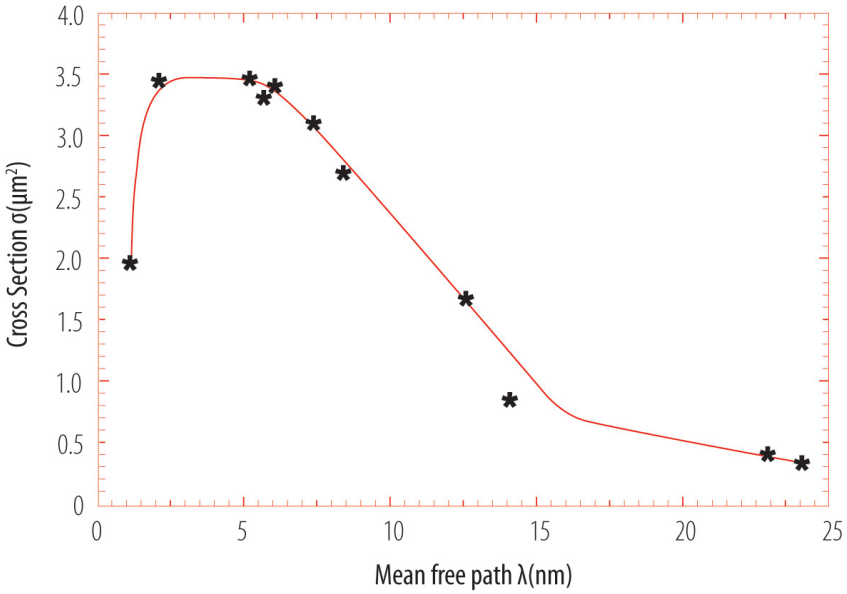

Figure 1: ***Inactivation cross section σ of V79 cells by protons versus the mean free path for primary ionization λ***

RESULTS AND DISCUSSION

The relation between the inactivation cross-section σ (μm^2) and the mean free path for primary ionisation λ (nm) is shown in Figure 1. A visual inspection of this curve can identify four regions: the first region, $\lambda \leq 2$ nm, and the second nearly flat region for $(2 \leq \lambda \leq 5)$ nm, followed by a decreasing cross section for $\lambda \geq 5$ nm up to $\lambda \approx 15$ nm, and finally the fourth region in which $\lambda > 15$ nm, which shows the lowest cross section values.

Before we discuss each of these regions, we must refer to Watt's results, in which he applied his model to heavy charged particles of all kinds including α-particles, in addition to three points concerning the inactivation of V79 cells by protons.[5] He obtained a nice grouping of data into two distinguished regions, one nearly flat region in which the data grouped around a horizontal straight line with a saturation cross section of about 38 μm^2, and a clear point of inflection at $\lambda \geq 2$ nm to lower cross section values in which the data grouped around a straight line with a gradient of about -1.22. The three proton data points are randomly distributed below the saturation region with cross section values of about a factor of ten less than that for other charged particles in this region.

Returning to Figure 1, we can say that regions 2 and 3 are similar in shape only to Watt's results for other charged particles, but with a lower cross section value in the saturation region which is about 3.5 μm^2. This result is about the same as that obtained by Watt [5] for protons i.e. about a factor of ten less than that for other charged particles; however, the inflection point occurs at $\lambda \approx 5$ nm instead of 2 nm.

The observation of a saturation region between $\lambda \geq 2$ and $\lambda \leq 5$ nm is certainly related to DNA damage. The geometrical cross-sectional area of the intra-molecular DNA is about (3.5- 4) μm^2. This cross-sectional area is consistent with the maximum observed value for protons that can penetrate one DNA segment along a mean chord trajectory through the nucleus. A double strand break will occur and the chance of repair is very small. In the case of low energy protons ($\lambda < 2$ nm), which are related to region 1 in Figure 1, the particle tracks have a projected range less than the mean chord length through the cell nucleus, hencegiving lower cross section values, in other words fewer double stranded DNA segments at risk and therefore an increased repair capability of the cell.

In region 3, the mean free path is certainly larger than the mean chord length, but still there is a possibility of interaction occurring at least with one strand of the DNA segment by one particle track and, if it happened that another particle track passed the DNA and made a break to the opposite strand of the DNA segment, then a double strand break will occur; of course, this possibility will decrease as λ increases.

In region 4, λ becomes large and the probability of occurrence of double strand breaks is negligible compared to single strand breaks, which means an in-

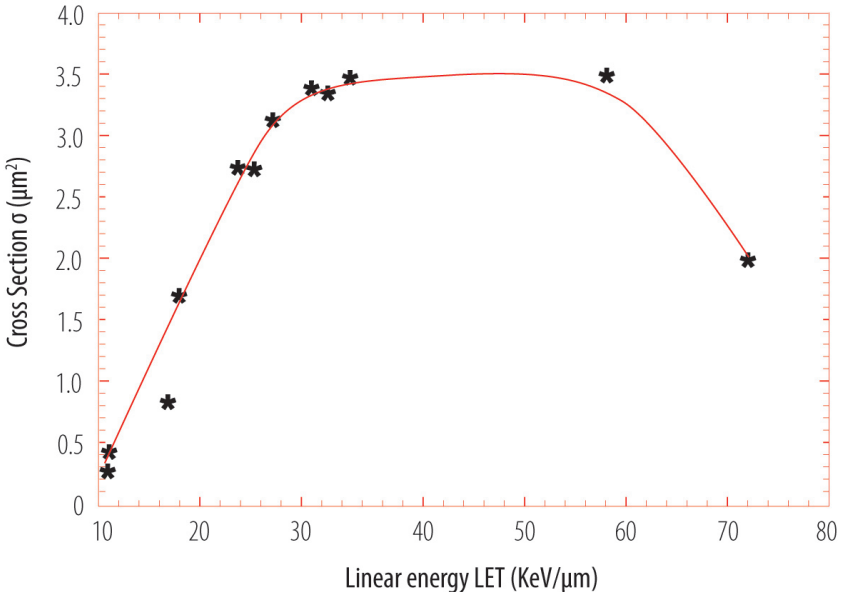

Figure 2: ***Inactivation cross section σ of V79 cells by protons versus the linear energy transfer LET***

creased repair capability of the cell.

The inactivation cross-section dependence on LET is shown in Figure 2. It is clear that there is a linear increase in cross section up to LET = 30 KeV/μm followed by a flat region between 30 and 60 KeV/μm

The lack of data points in this region makes it difficult to say if saturation occurs. The last point shows a decrease in cross section. At LET values below 30 KeV/μm, single strand breaks in the DNA segment take place. The maximum cross section value at LET ≈ 30 KeV/μm is compatible with what has been found in the RBE-LET relation by Belli [9] and Falkard [11] that the maximum damage for protons inactivation occurs at LET ≈ 24-27 KeV/μm.

CONCLUSION

In the inactivation of V79 cells by protons, the maximum inactivation cross section occurs when the mean free path for the incident proton is between 2 and 5 nm. In this region the inactivation cross section is about the same as the geometrical cross sectional area of the DNA in the cell. This means that one DNA segment is at a risk upon the traversal of one charged particle. The inactivation cross section is found to be 3.5 μm^2, this value is consistent with the geometrical cross sectional area of about 4.0 μm^2 for the intra-molecular DNA.

In the cross section versus LET curve, the maximum inactivation cross section occurs at LET = 30 KeV/μm. This value of LET is very close to that found by Belli[9] and Folkard[11] at which maximum relative biological effectiveness (RBE) values were found in the RBE-LET relationship.

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