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CLINICAL & BASIC RESEARCH

Phenomenological Explanation of Cell Inactivation Cross Section in Terms of Direct and Indirect Action

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تفسير ظاهري للمقطع العرضي لتعطيل الخلايا في ضوء التفاعل المباشر واللامباشر

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الملخص: **الهدف:** استخدام مُعطيات مُنحنيات البَقاء لتعطيل خلايا (V79) وخلايا (CHO–K1) بالبروتونات والنيوترونات وايونات (C12) و(He3) لدراسة دور التفاعل المباشر واللامباشر في تعطيل الخلايا. **الطريقة:** تمت دراسة منحنيات البقاء لخلايا (V79) المشععة بالبروتونات والنيوترونات وايونات (C12) وخلايا (CHO–K1) المشععة بايونات (He3) لمدى واسع من الطاقة باستخدام المصادر المنشورة. استخرجت النقاط العملية من منحنيات البقاء المنشورة باستخدام النظام الحاسوبي (Mat–lab) وأجريت عليها عمليات التوافق للمعادلة الخطية التربيعية. استخدمت معلمات التوافق لحساب المقطع العرضي للتعطيل (σ) عند الميل الاولي؛ والجرعة (2Gy)؛ وعند نسبة بقاء قدرها (10%) لكل جسيم على انفراد. **النتائج:** تُبيّن النتائج بصورة عامة أنّ المقطع العرضي للتعطيل يقل بشكل أسي تقريبا مع زيادة متوسط المسار الحر للتأين الابتدائي (λ)، عدا في حالة البروتونات، ولى حد ما النيوترونات، حيث يكون المقطع العرضي ثابتا عند قيم معينة ل λ. و يزداد المقطع العرضي بزيادة انتقال الطاقة الخطي (LET) و أيضا يُصبح غير معتمد على (LET) عند قيم معينة ل (LET). **الخلاصة:** تشير النتائج الى أنّ تحطيم الخلايا الذي يُعزى إلى انكسار قضيبي الحَمْضُ الرِّيْبِيُّ النَّوَوِي المَنْزُوع الأوكسِجين سببه الرئيسي هو التأثير اللامباشر والذي هو أكثر بكثير من التأثير المباشر.

مفتاح الكلمات: الحَمْضُ الرِّيْبِيُّ النَّوَوِي مَنْزُوع الأوكسِجين ، تعطيل الخلية، ميكانيكية الهدم، اشعاع.

ABSTRACT: ***Objectives:*** The aim of this study was to use survival curves data for the inactivation of V79 cells and CHO-K1 cells by protons, neutrons, C^{12} ions and He^{3} ions to study the role of direct and indirect action in cell inactivation. ***Methods:*** A large number of survival curves for the inactivation of V79 cells by protons, neutrons, and C^{12} ions and for CHO-K1 cells inactivated by He^{3} ions over a wide energy range were taken from published references. Experimental data points were extracted from the published survival curves using MATLAB (Version 7.0) and fitted to the linear quadratic equation. The fit parameters were used to calculate the inactivation cross section (σ) at the initial slope, the 2Gy dose and at 10% survival for each particle type separately. ***Results:*** The results, in general, showed that the inactivation cross section decreases nearly exponentially when increasing the mean free path for primary ionisation (λ), except in the case of protons, and to some extent neutrons, where the cross section takes a constant value at specific λ values. The cross section increased with increasing linear energy transfer (LET) and also became independent of LET at specific LET values. ***Conclusion:*** The results indicate that the cell damage due to the double strand breaks of DNA caused by indirect action is much larger than that caused by the direct action.

Keywords: Cell inactivation; Damage mechanism; Radiation; DNA.

Advances in Knowledge

1. *Indirect action is the main cause of damage to living cells.*
2. *Direct action is seen only in proton irradiation. This is what makes protons so effective in clinical radiotherapy.*

Application to Patient Care

1. *Differentiation between the direct and indirect action of cell inactivation helps to improve treatment methods for tumours.*
2. *Understanding inactivation mechanisms can help minimise the dose required when treating patients with radiation.*
3. *Accelerated protons and light ions have already proved their usefulness in clinical radiotherapy. To take the advantage of their possible benefits, and to optimise treatment procedures in individual cases, detailed understanding of the underlying radiobiological mechanism is necessary.*

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It is generally accepted that DNA is the principle target of radiation action and DNA double strand breaks (DSB) are the main cause of damage to living cells.[1,2] DNA damage may occur either via direct action in which particulate radiation strikes the DNA molecules directly, or via indirect action in which radiation interacts with the water molecules in the cell to form free radicals which in turn damage the DNA strands. Depending on the type of radiation dose, and the cells involved, the effects can occur relatively fast or may take years to be observed. It is believed that the direct damage mechanism accounts for a tiny proportion of biological effects caused by overall radiation.[3] Since direct observation of damage mechanism is impossible, physical quantities, such as inactivation cross sections, based on experimental observations are used to make prediction about these mechanisms. Watt *et al.* in a series of publications,[4-7] have shown that maximum damage occurs when the mean free path for primary ionisation λ matches the mean chord length (≈ 2 nm) in the DNA. In fact, very few researchers agree with this opinion.

The aim of this study was therefore to find out the role of direct and indirect action for protons, neutrons, He^3 ions and C^{12} ions.

Methods

Multiple survival curves measurements from two common cell lines: V79 (Chinese hamster) and CHO-K1 (Chinese hamster ovary) were used for this study. Published survival curves data for cell inactivation were taken from references,[8-18] scanned as image files and digitised to obtain the sets of experimental data points using the MATLAB computer program (MATLAB 7.0, The Mathworks, Inc., Natick, MA, USA). Forty seven survival curves (20 for protons, 13 for neutrons, 7 for He^3 ions and 7 for C^{12} ions) were fitted to the linear quadratic (LQ) equation using the curve fitting tool (cftool) facility in the MATLAB system.

The α and β values obtained from the fit were used to calculate the inactivation cross section σ. Although neutrons and other heavy ions are usually fitted to the linear term (α) of the LQ equation, we found that a better fit is obtained when fitting the data to both terms. The goodness of fit was measured depending on many statistical parameters and distributions. The criteria for the goodness of fit were that the data points coincided very well with the fitted curve; the residual distribution (residual = data – fit) was either symmetric around zero or the residual value for individual points was nearly zero; the adjusted R-square value (R-square is the best indicator of the fit and can take on any value between 0 and 1, with a value closer to 1 indicating a better fit) should be very close to 1, and the sum of squares due to error (SSE) should approach zero. The cross section relationships to the mean free path λ and the linear engery transfer (LET) were studied at three radiobiologically important points: initial slope of cell survival curve, 2Gy fraction dose typical for radiotherapy and at 10% survival dose for protons, neutrons, helium and carbon ions. The adjusted R-square values for each fit are shown in Tables 1 to 4.

Cross section calculations

The linear quadratic equation is given by:

$$\ln S = (-\alpha D - \beta D^2) \qquad (1)$$

Where S is the survival fraction, D is the dose and **α** and **β** are the fit parameters

Deriving this equation with respect to D represents:

$$\frac{-d\ln S}{dD} = \alpha + 2\beta D \qquad (2)$$

$\frac{-d\ln S}{dD}$ represents the slope of the survival at any dose D.

For the initial slope:

$$\lim_{D \to 0} \frac{-d\ln S}{dD} = \alpha \qquad (3)$$

The cross section is related to the slope of the survival curve as:[19,20]

$$\sigma(\mu m^2) = \frac{slope/Gy * L(keV/\mu m)}{6.25 * \rho(g/cc)} \qquad (4)$$

Where ρ is the density of the medium (g/cc) and L is the track average linear energy transfer in (kev/μm)

The α and β parameters obtained from the fit were used in equation (4) to calculate the cross section at:

a) the initial slope, slope = **α**

b) at 2Gy dose since it is relevant in radiotherapy

$$slope = \alpha + 4\beta$$

c) At 10% survival, this is usually used for data

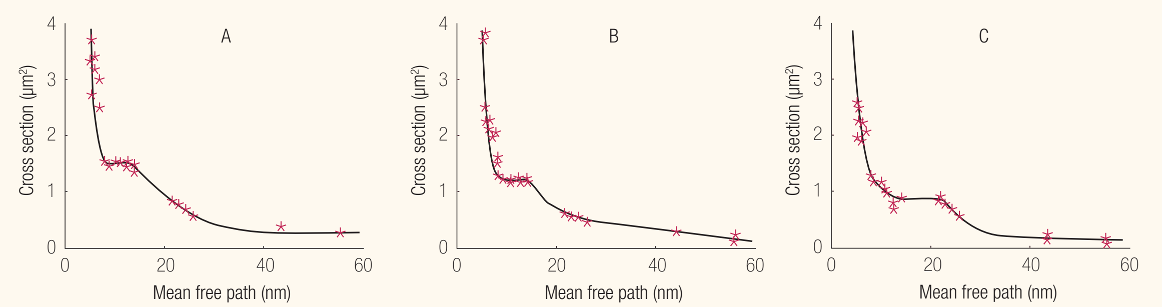

Figure 1: Inactivation cross section of V79 cells by protons versus mean free path: a) at the initial slope; b) at 2Gy dose; c) at 10% survival

comparison.

$$\text{slope} = \alpha + 2\beta D$$

where D represent the dose at 10% survival.

Results

The α and β values obtained from the fit and the calculated cross section values for each survival curve at the initial slope for protons, neutrons, He^3 ions, and C^{12} ions are presented in Tables 1 to 4.

σ – λ DEPENDENCE ON PARTICLE TYPE

The proton inactivation cross section σ of V79 cells, measured at the initial slope, 2Gy dose and at 10% survival is plotted against the mean free path λ in Figures 1a to 1c respectively. A distinguished feature of these figures is that the cross section decreases sharply below λ ≈ 8.7 nm and then takes a constant value at the initial slope and at 2Gy dose in the region between λ = (8.7–14) nm before it starts to decrease again at λ > 14 nm, but this flat region has nearly vanished at 10% survival.

The neutron cross section σ versus the mean free path λ in the same regions selected for protons is plotted in Figures 2a to 2c in the same order as above. A sharp decrease of cross section is seen in all these figures. It is nearly exponential in shape, but still one can notice a very small flat region at the initial slope [Figure 2a] which lies between (1.56< λ<2.5) nm.

In general, the proton and neutron cross section at 2Gy dose are somewhat higher than those at the initial slope.

The inactivation cross section of CHO-K1 cells by He^3 ions at the initial slope, 2Gy dose and at 10% survival is shown in Figures 3a to 3c respectively, and the corresponding figures for the inactivation of V79 cells by C^{12} ions are shown in [Figures 4a to 4c]. Both groups of figures show that the cross section decreases in an exponential shape without any structure. The cross section values are very close to each other in the three selected regions.

σ – LET DEPENDENCE ON PARTICLE TYPE

The inactivation cross section for the particles involved in this study is plotted against the linear energy transfer LET in the same regions of the survival curve mentioned above.

For protons, the cross section at the initial slope [Figure 5a] and at 2 Gy dose [Figure 5b] increases nearly linearly with LET up to LET = 16.8 keV/μm,

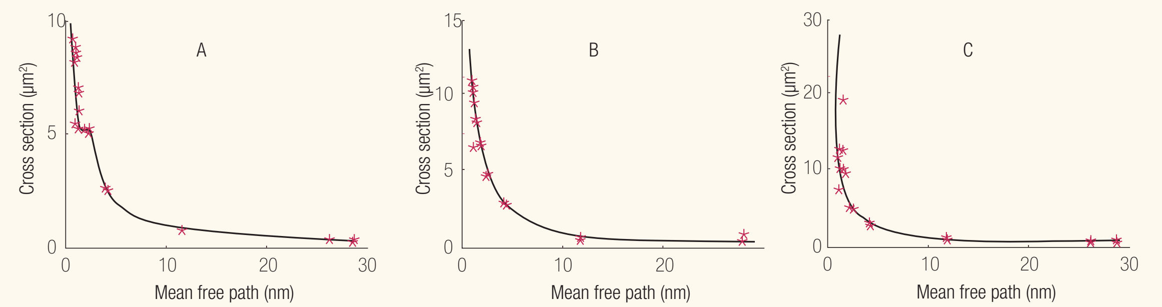

Figure 2: Inactivation cross section of V79 cells by neutrons versus mean free path: a) at the initial slope; b) at 2Gy dose; c) at 10% survival.

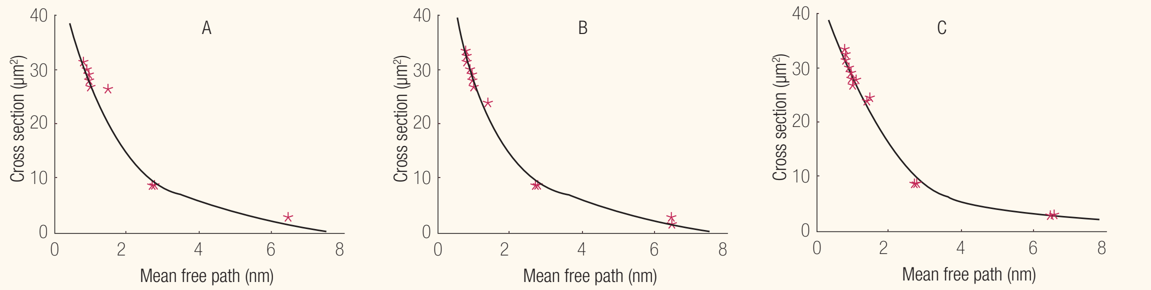

Figure 3: Inactivation cross section of CHO-K1 cells by 3He ions versus mean free path: a) at the initial slope; b) at 2Gy dose; c) at 10% survival.

then takes a constant value between LET = 16.8 keV/μm and LET = 23.6 keV/μm, followed by a linear increase. The flat region is not very clear at 10% survival [Figure 5c].

A similar behaviour is also seen for neutrons, where the flat region occurs between LET = 32.4 keV/μm and LET = 48 keV/μm [Figures 6a at the initial slope and Figure 6b at 2Gy dose]. Again the flat region is not very clear at 10% survival [Figure 6c]. In the case of He^3 ions, the cross section increases up to LET =133 keV/μm then starts to decrease sharply. This is the case for all regions under consideration in this study [Figures 7a to 7c].

The inactivation cross section for C^{12} ions increases up to LET = 339.1 keV/μm at the initial slope [Figure 8a] and then starts to decrease very slowly, but it reaches saturation at this value of LET in the 2Gy dose [Figure 8b] and the 10% survival [Figure 8c].

Discussion

It is believed that DSB are the main cause of cell damage and this damage is either due to direct action, in which the energy is directly deposited in the target molecule of biological interest, without the intervention of radical species derived from water analysis, or due to indirect action which is defined as the outcome of the radiolytic products of water on the target of biological importance.

As mentioned above, the inactivation cross section decreases with an increasing mean free path λ. This decrease takes an exponential shape in the case of He^3 ions [Figure 3a to 3c] and C^{12} ions [Figure 4a to 4c], but the exponential shape is interrupted by a flat region in the case of protons [Figure 1a to 1c] and to a lesser extent neutrons [Figure 2a to 2c]. The cross section values at their maximum are about 3.5, 10, 30, 40 μm^2 for protons, neutrons, He^3 ions and carbon ions respectively. These values are not outside the range of cross section values found by Watt[21] and Belloni *et al.*[22] The difference in cross section values for different particles is due to the dependence of ionisation density on particle type. For example, Tables 1, 2, and 3 that: 1.07 MeV protons have LET = 27.6 keV/μm, while 1 MeV neutrons have LET = 32.39 keV/μm, and 1.08 MeV He^3 ions have LET = 138 keV/μm. This means that neutrons are able to produce more secondary particles than protons of the same energy and this applies to He^3 ions and C^{12} ions. The result is higher cell damage or a higher cross section.

Usually the exponential shape refers to random events such as the exponential decay law. Assuming

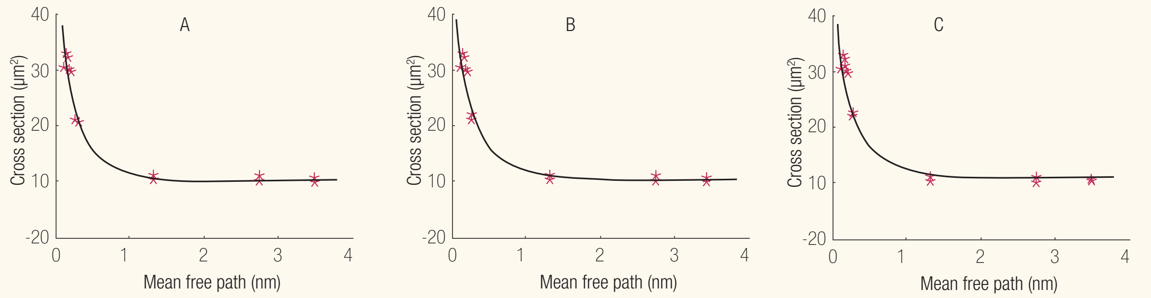

Figure 4: Inactivation cross section of V79 cells by 12C ions versus mean free path: a) at the initial slope; b) at 2Gy dose; c) at 10% survival.

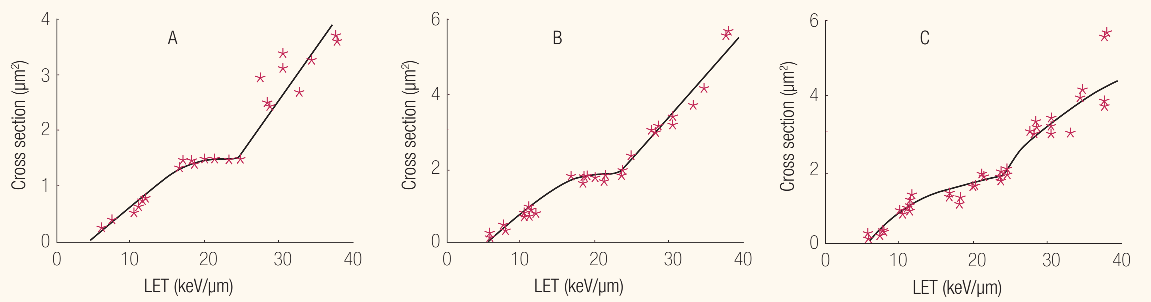

Figure 5: Inactivation cross section of V79 cells by protons versus linear energy transfer: a) at the initial slope; b) at 2Gy dose; c) at 10% survival.

that the indirect action of radiation is a stochastic process in which single strand breaks (SSB) happen by the effect of secondary radicals produced at random along the particle track, these radicals are highly reactive and are able to change the structure of a DNA biologically important molecule (a molecule that carries genomic information), and this may lead to cell inactivation. A radical can hit a single strand of DNA only and there is a probability that the SSB may undergo a repair process, but, since these radicals are large in number, it is very probable that the other strand is hit by another radical leading to DSB which is the main cause of damage to living cells. The breakage of each strand of the DNA separately is a totally random process and, as mentioned before, random processes take an exponential shape depending on the action that causes them. In this case, it is the strength of the ionisation density. This phenomenology applies to He^3 ions [Figures 3a to 3c] and C^{12} ions [Figures 4a to 4c] at the three regions of interest in this study since these particles are highly ionising radiations and are able to produce a large numbers of free radicals.

Protons and neutrons at low λ values have large ionisation density and are able to produce many radicals, but not as large as that for heavier ions. This is reflected in the lower cross section values at the lower end of the $\sigma - \lambda$ curve for each of them. So the cell inactivation by protons up to λ= 8.7 nm and that for neutrons up to λ= 1.56nm is dominated by indirect action. The small flat region between (8.7≤ λ≤ 14) nm for protons [Figure 1a] and between (1.56< λ<2.5) nm for neutrons [Figure 1b] means a different mechanism is in action. We think it is the direct action process in which the two DNA strands are hit by the incident particle itself at the same time and not by its secondary radicals. Observing a flat region at these specific values of λ means that there is some kind of relation between λ and the DNA structure which enhances the direct action. This relation is not yet well understood and needs more investigations. The reasons for this observation of the direct action of protons and not observing it in the case of neutrons, He^3 ions, and C^{12} ions is that the competitiveness between direct and indirect action for cells inactivated by protons is not as large as that for cells inactivated by one of the other three particles under study because of the small indirect action cross section in the case of protons compared to that for He^3 ions, C^{12} ions and neutrons. The direct action of heavy ions, such as He^3 ions and C^{12} ions, is missed because of the large indirect action. Moreover, the high λ- edge of the

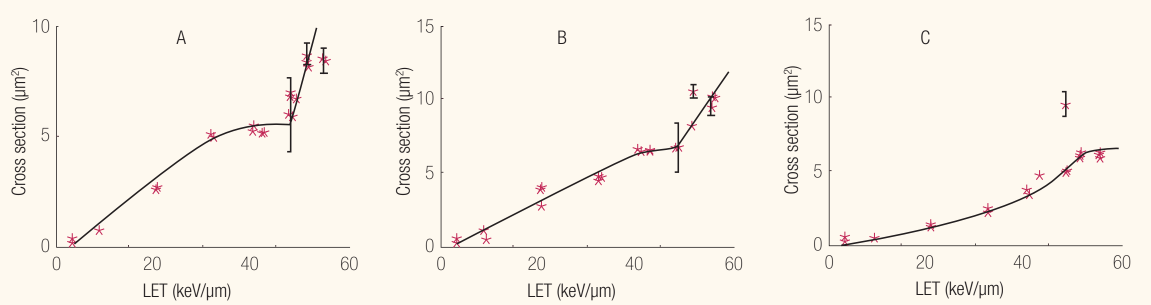

Figure 6: Inactivation cross section of V79 cells by neutrons versus linear energy transfer: a) at the initial slope; b) at 2Gy dose; c) at 10% survival.

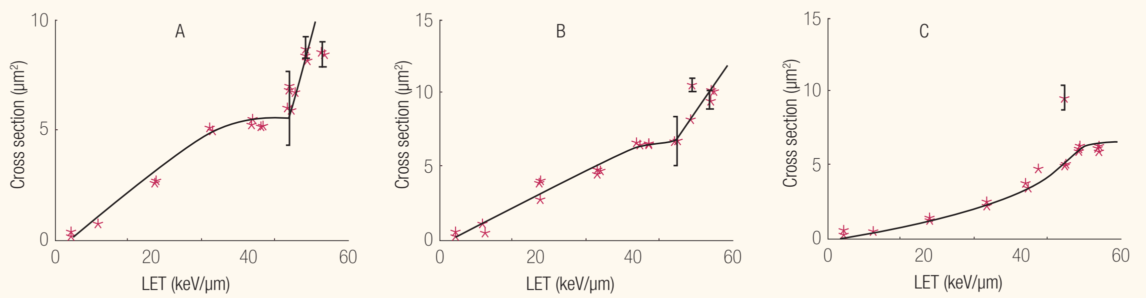

Figure 7: Inactivation cross section of CHO-K1 cells by 3He ions versus linear energy transfer: a) at the initial slope; b) at 2Gy dose; c) at 10% survival.

proton curve is not asymptotic as for other particles; this means that there are still some direct action events taking place. These observations explain why protons have a wide usage in radiotherapy and are regarded as having the ability to concentrate the dose in more discrete target volumes. They would deposit all their energy in the cells of the target, without irradiating normal cells that may be in the target since they produce less indirect effects which are randomly distributed.[23]

The proton inactivation cross section at 2Gy dose [Figure 1b] shows similar behaviour to that at the initial slope and the flat region can still be seen since protons, unlike other particles, are not heavily ionising. Hence they produce a lower number of radicals, but the flat region in the case of neutrons at 2Gy dose [Figure 2b] has vanished because neutrons are heavy ionising particles compared to protons and are able to produce more radicals which enhance the indirect action and cause the direct action effect to disappear. The higher cross section values obtained at the low λ edge of the σ – λ curve for protons and neutrons at 2Gy dose as compared to those at the initial slope, are because of the higher number of secondary radicals produced by a 2Gy dose than lower doses.

The flat region in the proton inactivation cross section at 10% survival [Figure 1c] is not as clear as that at the initial slope or at 2Gy dose. This is because that the 10% survival may occur at any dose larger than one gray depending on the energy of the particle, so we expect a large mixture of direct and indirect action in this region, but the direct action still plays a major role in the inactivation of cells. The neutron cross section at 10% survival [Figure 2c] shows an exponential shape which means that the indirect action dominates.

The direct–indirect action mechanism is seen also in the σ – LET relationship for protons [Figures 5a to 5c]. At low LET values, which correspond to the high λ-edge of the σ – λ curve, the increase of cross section with increasing LET up to LET=16.8 keV/μm indicates the dominance of indirect action and the flat region at (16.8 ≤ LET ≤ 23.6) keV/μm, which corresponds to (14 ≥ λ ≥ 8.7) nm, is attributed to direct action, where it occurs purely in this specific region. Then, the cross section increases nearly linearly with increasing LET to resume the indirect action phase.

The same thing can be said about the neutron cross section at the initial slope [Figure 6a], but there are very few data points in the flat region since there is not much published data for the inactivation of V79 cells by neutrons. The flat region is better

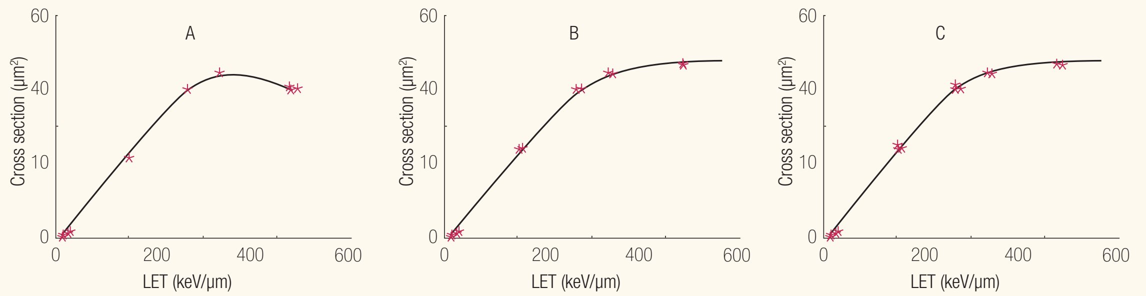

Figure 8: Inactivation cross section of V79 cells by 12C versus linear energy transfer: a) at the initial slope; b) at 2Gy dose; c) at 10% survival.

Table 1: Survival curve parameters for the inactivation of V79 cells by protons

E (MeV)	LET (kev/μm)	λ (nm)	α (Gy-1)	β (Gy-2)	σ (μm2)	R^2	Reference
0.625	37.8	5.5	0.995	--------	3.7	0.9989	Belli[8]
0.7	34.6	5.7	0.89	0.048	3.31	0.9981	Belli[8]
0.73	33.15	5.71	0.73	--------	2.74	0.9991	Belli[9]
0.826	30.5	6.14	0.86	0.0073	3.2	0.9996	Belli[8]
0.84	30.4	6.24	0.914	--------	3.4	0.9942	Belli[9]
0.92	28.5	6.8	0.80	0.089	3.0	0.9972	Paganetti[10]
1.07	27.6	7.16	0.801	0.033	2.98	0.9992	Folkard[18]
1.16	23.6	8.7	0.39	0.06	1.45	0.9989	Belli[9]
1.41	21.2	10.2	0.401	0.095	1.52	0.9985	Paganetti[10]
1.5	20	11.18	0.419	0.039	1.56	0.999	Belli[8]
1.7	18.3	12.58	0.403	0.065	1.5	0.998	Belli[9]
1.71	18.26	12.66	0.395	0.0006	1.47	0.9967	Paganetti[10]
1.9	16.8	14	0.382	0.076	1.42	0.9998	Folkard[11]
3	11.9	22	0.218	--------	0.81	0.9835	Paganetti[10]
3.2	11.7	22.9	0.212	0.32	0.79	1.0000	Schettino[12]
3.36	11.3	23.99	0.193	0.03	0.72	0.9992	Belli[9]
3.5	11	24.8	0.18	0.049	0.67	0.9991	Belli[8]
3.66	10.6	25.9	0.148	0.048	0.55	0.9994	Paganetti[10]
6	7.7	43.6	0.105	0.166	0.39	0.9991	Belli[8]
7.4	5.87	55.5	0.062	0.0128	0.23	0.9281	Paganetti[10]

Legend: LET = linear energy transfer

seen in the σ – LET curve for neutrons at 2Gy dose [Figure 6b] and at 10% survival [Figure 6c] than that in the σ – λ curve.

No flat region can be seen in σ – LET curve for He^3 ions [Figures 7a, 7b and 7c) and C^{12} ions [Figure 8a, 8b and 8c] since the direct action is negligible compared to the indirect action, but the cross section increases nearly linearly to reach a maximum value at LET = 133 keV/μm ≡λ= 0.82 nm in He^3 ions. It is less obvious, but can still be distinguished to have its maximum at LET = 339.1keV/μm ≡λ=0.13 nm in C^{12} ions at the initial slope and takes a saturation shape at 2Gy dose and 10% survival. These peaks or saturations indicate that an overkill process is taking place.

Conclusion

Inactivation cross section due to ionising radiations provides a good tool to study damage mechanisms since it takes different shapes for different particles when plotted against physical parameters as the mean free path or the LET.

In this study, we have found that the inactivation

Table 2: Survival curve parameters for the inactivation of V79 cells by neutrons

E (MeV)	LET (kev/µm)	λ (nm)	α (Gy-1)	β (Gy-2)	σ (µm2)	R^2	Reference
0.11	51.64	1.21	0.82	0.28	8.67	0.9968	Hall[13]
0.176	55.32	1.23	0.804	0.003	8.5	0.9995	Leenhout[14]
0.22	55.06	1.266	0.795	0.21	8.4	0.9988	Hall[13]
0.34	51.2	1.43	0.776	0.15	8.2	0.9999	Hall[13]
0.43	48.15	1.55	0.653	0.26	6.9	0.9995	Hall[13]
0.433	48.02	1.56	0.558	0.15	5.9	0.9955	Leenhout[14]
0.583	42.74	1.8	0.485	0.11	5.13	0.996	Leenhout[14]
0.66	40.53	1.26	0.501	0.136	5.3	0.9995	Hall[13]
1	32.39	2.5	0.47	0.058	5.0	0.9995	Hall[13]
2	20.7	4.317	0.24	0.022	2.55	0.9999	Hall[13]
6	9.05	11.72	0.06	0.057	0.65	0.9989	Hall[13]
13.6	3.7	26.16	0.02	0.39	0.23	0.9986	Hall[15]
15	3.4	28.84	0.016	0.05	0.17	0.9994	Hall[13]

Legend: LET = linear energy transfer

cross section σ in general decreases with increasing mean free path λ; this decrease takes an exponential shape in the inactivation of CHO-K1 cells by He^3 ions and V79 cells by C^{12} ions. The exponential shape is attributed to the interaction of secondary radicals with DNA to produce DSB which are the main cause of cell damage. This process is described as an indirect action process. The inactivation cross section of V79 cells by protons and neutrons also decreases nearly exponentially with increasing λ and this decrease is due to indirect action, but the semi-exponential shape in the case of protons, and to some extent neutrons, is interrupted by a flat region at specific λ-values. We believe this flat region is due to the interaction of the incident particle itself with the DNA strands, and the process is a direct action process. This gives protons the ability to concentrate the dose in more discrete target volumes which is very useful in radiotherapy treatments.

The σ-LET curves for the particles under study have shown that the cross section increases nearly linearly with LET and the flat region is also seen in

Table 3: Survival curve parameters for the inactivation of CHO-K1cells by He3 ions

E (MeV)	LET (kev/µm)	λ(nm)	α (Gy-1)	β (Gy-2)	σ (µm²)	R square	Reference
0.84	133	0.82	1.487	----------	31.6	0.9995	Muller[16]
1.13	138	0.92	1.359	----------	29.5	0.9994	Muller[16]
1.22	140	0.95	1.245	0.00004	27.9	0.9999	Muller[16]
1.38	142.6	0.988	1.23	----------	28.06	0.9992	Muller[16]
2.3	121.5	1.46	1.24	----------	26.8	0.9997	Muller[16]
4.6	77.8	2.78	0.684	----------	8.7	0.9953	Muller[16]
10.7	41	6.5	0.284	0.030	2.6	0.9991	Muller[16]

Legend: LET = linear energy transfer

Table 4: Survival curve parameters for the inactivation of V79 cell by carbon ions

E (MeV)	LET (kev/µm)	λ(nm)	α (Gy-1)	B (Gy-2)	σ (µm²)	R^2	Reference
2.4	339.1	0.13	0.84	--------	44.5	0.9989	Weyrather[17]
4.2	482.7	0.15	0.52	0.021	40.16	0.9907	Weyrather[17]
5.4	275.1	0.18	0.91	--------	40.0	0.9933	Weyrather[17]
11	153.5	0.26	0.87	0.0549	21.5	0.9991	Weyrather[17]
76.9	32.4	1.32	0.255	0.04	1.3	0.9963	Weyrather[17]
190.7	16.8	2.75	0.22	0.02	0.59	1	Weyather[17]
266.4	13.7	3.47	0.139	0.05	0.3	0.9988	Weyrather[17]

Legend: LET = linear energy transfer

the proton and neutron cross section, but not in He^3 ions or C^{12} ions.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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