Direct comparison between protons and alpha particles of the same LET

Background:

High LET more effective than low LET…well established in…

- Cell survival
- Mutagenesis
- Chromosome aberrations

Systematic studies of the same particle type with a wide range of LETs are difficult.

- Ranges are short.
- High particle energies required to reach lower LET values.
- Important for mechanistic studies of biological effects.

RBE *vs* LET
Barendsen….classic graph

- Widely used to interpret high-LET effects.
- Has influenced selection of radiation protection weighting factors.
- Barendsen used a mixture of x-rays, deuterons and alpha particles.

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[Barendsen, 1968]

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Other workers have partially reproduced the Barendsen data.

- Blakeley used very high LET particles
- None used protons > 11 keV/μm
- All used a mix of particles.

e.g., Bird et al., "Inactivation of Synchronized Chinese Hamster V79 Cells with Charged Particle Track Segments", Rad. Res., 82, 277-289, 1980.

TABLE I

Physical Characteristics of the Charged Particles

Ion	Accelerated energy (MeV)	LET (keV/μm)	
		Track average	Entrance—6-μm depth
Proton	4.0	10.2	10.1— 10.3
Deuteron	3.3	20.4	20.2— 20.6
	2.2	31	30 — 32
	1.9	40	37 — 43
Helium-3	7.9	67	65 — 69
	6.3	91	87 — 96
	5.0	127	116 —143
	4.6	170	142 —219

TABLE III

RBE Values as a Function of LET

LET (keV/μm)	Late-S cells			G_1/S cells		
	α/α_x	D_x/D (1%)	D_x/D (0.2%)	α/α_x	D_x/D (1%)	D_x/D (0.2%)
10	1.4	1.1	1.1	1.8	1.1	1.1
20	2.3	1.5	1.4	2.3	1.5	1.5
31	3.0	1.7	1.6	3.5	1.9	1.9
40	4.0	2.0	1.9	3.9	2.1	2.3
91	8.0	2.4	2.2	7.1	2.6	2.6
127	9.1	2.7	2.4	6.7	2.6	2.6
170	7.6	2.1	1.9	6.5	3.0	3.0

It was commonly assumed that protons and alphas of the same LET **lie on the same smooth curve**.

Yet, track structure differences were predicted ("subtle differences").

✯ These common assumptions were challenged by a report from Belli et al.,

"RBE-LET relationship for the survival of V79 cells irradiated with low energy protons". Int. J. Radiat. Biol., 55, 93-104, 1989.

- Cells on mylar film
- Protons with LETs ranging from 10.6 – 34.5 keV/μm
- Survival curves showed increasing "linearity"

Dose calculated as D (Gy) = 0.16 (F)(L)

Where F= proton fluence (μm^{-2})

And L = LET keV/μm

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[Belli, 1989]

Calculated RBEs relative to x rays were larger than RBEs *reported in the literature* for alphas of the same LET.

Complications:
Survival curves had "tails" at the highest LET values, the *two most critical points*.
Attributed to cells that received no dose (or a very reduced dose) due to:

- Rounding up, or actual detaching, of the cell from the membrane (?)
- Elevated cells (?)
- Partial shielding of cells at the edge of the dish (?)

Tested by washing and not harvesting cells near the edge of the dish.
Seemed to improve the data, but not shown….

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Table 2. Parameters obtained from best fits of the V79 survival curves.

Radiation	$\alpha(Gy^{-1})$	$\beta(Gy^{-2})$	f	α/α_x
X-rays	0·128 ± 0·023	0·046 ± 0·005	—	1·0
Protons				
10·6 keV/μm	0·378 ± 0·031	0·040 ± 0·009	—	3·0
17·8 keV/μm	0·586 ± 0·052	0·037 ± 0·016	—	4·6
23·9 keV/μm	0·938 ± 0·019	—	—	7·3
30·4 keV/μm	0·803 ± 0·059	—	0·061 ± 0·007	6·3
34·5 keV/μm	0·536 ± 0·040	—	0·189 ± 0·015	4·2

Implications:

- Protons have a higher RBE than alphas at the same LET

- On the RBE *vs* LET plot, the protons "peak" at ~ 20 keV/μm whereas, the alphas (and everything else) peaks at about 100-200 keV/μm.

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[Belli, 1989]

This was surprising. The differences in track structure are "subtle".

Protons and alpha articles in these energy ranges are of particular interest:

- Neutrons, which are of great concern in radiation protection, produce low energy recoil protons in tissue: in the 10-90 keV/μm range.

- Radionuclide sources used in therapy and radon produce "slow" alpha particles: in the 60-250 keV/μm range.

- Protons are used in therapy at the Bragg peak. A significant proportion of the dose is delivered by low energy protons.

The series of papers by Goodhead et al., working with Belli, was a thorough study of both proton and alphas by the same group using the same endpoint and same irradiation facilities. The experiment was extended to additional endpoints, mutation, DSB, to investigate the extent of this phenomenon.

What ***ARE*** the differences between 1.2 MeV protons and 30.5 MeV alpha particles?

	1.2 MeV proton	***30.5 MeV alpha***
LET	23 keV/μm	23 keV/μm
Velocity	1.5×10^7 m/s	3.8×10^7 m/s
Range in water	30 μm	730 μm
Max E trans. to e^-	2.6 keV	16.6 keV
Core radius	0.0006 μm (6 Å)	0.0015 μm (15 Å)
Penumbra radius	0.070 μm	1.76 μm

INT. J. RADIAT. BIOL., 1992, VOL. 61, NO. 5, 611-624

Direct comparison between protons and alpha-particles of the same LET: I. Irradiation methods and inactivation of asynchronous V79, HeLa and C3H 10T½ cells

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Objective: direct comparison of protons and alphas in the LET range reported by Belli to have different RBEs

- Variable energy cyclotron
- 4 different beams: protons and alpha particles at 20 keV/μm and 23 keV/μm.
- This is the range in the Belli report where the LET differences are substantial,
- The particle ranges are still large enough to not cause serious experimental difficulties.
- Irradiation of cells in "track segment" mode.

Table 1. Beam characteristics

Session no./Beam designation[a]	Particles	Nominal machine energy (MeV)	Gold scattering foil (mg cm^{-2})	Energy at cell[b] entrance (MeV)	LET at cell[c] entrance (keV μm^{-1})	Approx.[d] dose rates (Gy min^{-1})		Residual range (μm)[e]
						In wheel	Static	
1 p(x)	Protons	3·2	2·68	1·00	25·38	1·6	120	24
2 α(1)	Alphas	39·97	24·17	34·87	20·63	1·5	110	952
3 p(1)	Protons	3·37	2·68	1·42	19·84	1·6	110	43
4 p(2)	Protons	3·26	2·68	1·22	22·13	2·0	150	33
5 α(2)	Alphas	35·45	24·17	30·53	22·96	1·1	80	752
6 p(1)rpt	Protons	3·37	2·68	1·46	19·49	1·8	130	45
7 p(2)rpt	Protons	3·26	2·68	1·24	21·92	2·2	160	34
8A α(2)rpt	Alphas	35·45	24·17	30·41	23·04	1·5	110	747
8B α(1)rpt	Alphas	39·97	24·17	35·66	20·26	1·6	—	991

[a] (1) and (2) indicate nominal LET ≈ 20 and 23 keV μm^{-1}, respectively; rpt indicates a repeat experiment. Sessions 8A and 8B were on the same day.
[b] Measured energy at the entrance surface of the monolayers of cells.
[c] LET in water, at the entrance surface of the cells, corresponding to the measured energy (Ziegler 1977, Janni 1982, Stevens *et al.* 1989).
[d] Approximate dose-rates only are quoted here because the beam currents varied slightly during the shifts.
[e] Residual range in water from the entrance surface of the cells (Ziegler 1977, Janni 1982, Stevens *et al.* 1989).

Table 2. Energies and LETs of the beams

Nominal LET (keV μm⁻¹)	Particles	Beam	At cell entrance: Energy ± HWHH (MeV)[a]	At cell entrance: LET ± HWHH (keV μm⁻¹)[b]	At 3 μm depth: Energy (MeV)[b]	At 3 μm depth: LET (keV μm⁻¹)[b]
20·3	Alphas	α(1)	34·87 ± 0·26	20·63 ± 0·12	34·81	20·66
		α(1)rpt	35·66 ± 0·25	20·26 ± 0·12	35·60	20·29
	Protons	p(1)	1·42 ± 0·08	19·84 ± 0·79	1·36	20·46
		p(1)rpt	1·46 ± 0·07	19·49 ± 0·68	1·40	20·08
23·0	Alphas	α(2)	30·53 ± 0·29	22·96 ± 0·18	30·46	23·01
		α(2)rpt	30·41 ± 0·31	23·04 ± 0·18	30·34	23·08
	Protons	p(2)	1·22 ± 0·08	22·13 ± 1·02	1·15	23·04
		p(2)rpt	1·24 ± 0·08	21·92 ± 1·00	1·17	22·79

[a] Measured energy at the entrance surface of the monolayers of cells ± half-width at half-height of the measured spectrum.
[b] Calculated, in water, from the measured energy and the stopping power and range tabulations of Stevens *et al.* (1989) based on the proton data of Janni (1982) and α-particle data of Ziegler (1977).

Table 3. Doses recorded on CR39 track-etch discs

Beam	Full or reduced beam	Number of wheel revolutions[a]	Monitor recorded dose (cGy)	Mean track density × 10² (μm⁻²)	CR39 Recorded dose[b] (cGy)
α(1)	F	2	5·63	1·67	5·51
				1·61	5·32
	R	8	2·56	0·792	2·61
p(1)	F	2	7·26	2·44	7·74
	R	8	3·02	0·780	2·48
p(2)	F	2	8·06	2·07	7·33
	R	8	2·57	0·613	2·17
α(2)	F	2	5·04	1·42	5·21
	R	8	1·72	0·480	1·76
p(1)rpt	F	2	7·36	1·90	5·93
	R	8	2·61	0·747	2·33
p(2)rpt	F	2	7·80	2·41	8·46
	R	8	2·43	0·529	1·86
α(2)rpt	F	2	6·24	1·71	6·31
	R	8	1·54	0·447	1·65
α(1)rpt	F	2	5·27	1·61	5·22
	R	8	1·79	0·544	1·76

[a] For comparison all biological irradiations were with full beams and large numbers of revolutions (typically ~16 revolutions per Gy).
[b] Calculated as $D = LF$ where L is the LET of the beam in water and F is the fluence (i.e. mean track density).

Beam path after leaving the vacuum of the accelerator beam line:

- 21.1 mm air
- 2.5 μm Hostaphan foil (0.36 mg/cm^2)
- cells (V79 cells ~ 4 μm-thick)

Aim is to match LET at 3 μm depth in the cells.

Dosimetry used

- Thin window ionization chamber
- CR39 track etch detectors
- Detectors in exact position as the cells

Fairly good agreement between the two dosimetry methods.
(Proton dose at low energy lower in CR39 possible due to poor penetration of the protons into the track etch detector.)

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Cell Survival Curves

Cells irradiated in a horizontal beam..”dry” briefly.

Cells were cut out on the Hostaphan film, *leaving a 2mm edge strip unused*.

Survival curves fit with the linear quadratic equation

$-\ln S = \alpha D + \beta D^2$

Dose = (LET)(track density)

[Image removed due to copyright considerations]

All cells irradiated as **exponentially growing** monolayers.

Table 4. Fitted parameters for cell inactivation

Cell line	LET (keV μm^{-1})	Particles	Linear coefficient a (Gy^{-1})	Dose^2 coefficient b (Gy^{-2})	Ratio of linear coefficients B
V79	20·3	Protons	0·42±0·05	0·019±0·008	1·69±0·42
		Alphas	0·25±0·06	0·043±0·008	
	23·0	Protons	0·30±0·06	0·052±0·008	1·43±0·37
		Alphas	0·21±0·04	0·047±0·005	
HeLa	20·3	Protons	0·53±0·12	0·084±0·025	1·26±0·36
		Alphas[a]	0·42±0·07	0·080±0·017	
	23·0	Protons	0·85±0·16	0·037±0·028	1·31±0·27
		Alphas	0·65±0·06	0·059±0·010	
HeLa S3	20·3	Protons[a]	0·67±0·12	0·037±0·023	0·94±0·27
		Alphas[a]	0·71±0·16	0·013±0·036	
	23·0	Protons	1·01±0·11	−0·001±0·021	1·28±0·15
		Alphas	0·79±0·04	0·006±0·007	
C3H 10T½	20·3[a] and 23·0[a]	Protons	0·43±0·07	0·013±0·021	0·91±0·18
		Alphas	0·48±0·05	0·014±0·016	

[a] Single experiment for each of these cases.

RBE calculation

- B defined as the ratio of the effectiveness of protons/alphas…at the same LET.
- At low doses B = ratio of the α linear dose coefficients.
- B=1.69 and 1.43 for the V79 experiments
  - Significantly greater than 1
  - In agreement with Belli, 1989 (also used V79 cells)
  - Belli calculated the RBEs the same way (ratio of α linear dose coefficients)
- RBE also significantly > 1 for HeLa cells, but not the C3H 10T½ cells.
- Cell thickness did vary somewhat, but the range of the highest LET proton (1.22 MeV) was still 33 μm.

Conclusions:

- Protons are more effective than alpha particles at the same LET.
- The effect was largest for V79 cells.
- Results support the conclusions of Belli, 1989.

Sugggestion…

- Limited range makes experiments difficult.
- Use deuterons to increase range?
- At the same velocity, deuterons have the same LET and track structure as protons, **but twice the range**.

Direct comparison of biological effectiveness of protons and alpha-particles of the same LET. II. Mutation induction at the HPRT locus in V79 cells

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Objective: RBE differences observed in cell survival experiments by these investigators between alphas and protons of the same LET. *Extend these observations to additional endpoints.*

HPRT Gene Mutation assay: HPRT is hypoxanthine-guanosine phosphoribosyl transferase. This enzyme is responsible for putting purine bases on the phospho-ribose structure. *A key enzyme in DNA synthesis*.

Methods:

- Doses used produce a survival of >10%
- Cells plated in the presence of 6-thio-guanine, a purine analog.
- 6-thio-guanine is toxic to cells if incorporated into DNA.
- HPRT will accept the 6-thioguanine as a substrate and transfer to phosphoribose.
- Only cells that have an **inactive** HPRT will survive this "selection pressure".

Mutation is a rare event.

Need an assay to **select** mutants from very large numbers of cells.

Requires a hit in a specific DNA sequence, the HPRT gene.

Requires the cell to survive.

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Data plotted as #colonies/10^5 cells *vs* dose.

$$M = \alpha D$$

where M = mutation frequency, α is the slope, D is the dose.

The ratio of the slopes used to calculate the RBE between alphas and protons directly (no x-rays involved.)

Table 1. Parameters obtained from the best fits of the V479-4 mutation curves

LET	Particles	$(\alpha \pm SE)10^5$ (Gy^{-1})	$(\sigma \pm SE)10^5$ (μM^2)	$\alpha_p/\alpha_\alpha \pm SE$
20 keV/μM	Protons	1·87±0·09	6·0±0·3	1·85±0·31
	Alphas	1·01±0·16	3·2±0·5	
23 keV/μM	Protons	1·99±0·14	7·3±0·5	2·07±0·19
	Alphas	0·96±0·06	3·5±0·2	

Mutation cross section:
Interpreted as the geometrical area of the target times the probability that a track will cause a mutation.

$$\sigma = \frac{0.16 L \alpha}{\rho}$$

σ is the cross section in μm^2
α is the slope (Gy^{-1})
L is LET (keV/ μm)

- Cross sections are larger for protons compared to alphas by a factor of ~2!!
- I.e., for each "hit" the proton is 2 times more likely to cause a mutation.
- No variation with LET (not surprising, since the difference is only 20-23!!)

RBE for HPRT mutation

- Calculated as the ratio of the slopes in the mutation induction graphs
- RBEs are ~2
- No variation with LET

Mutation frequency vs cell inactivation.

Protons are more mutagenic than alpha particles, at the same level of survival.

[Image removed due to copyright considerations]

Relation to previous studies:

- Alpha particles at 90-130 keV/μm have 6-9 times higher mutation frequency than the current study.
- Protons in the 90-130 keV/μm range not studied yet.
- Belli states "underway" in 1992. Not in the literature…
- Difficult to get protons to this range of LET

Direct comparison of biological effectiveness of protons and alpha-particles of the same LET.
III. Initial yield of DNA double-strand breaks in V79 cells

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Objective: Extend the study to another important cellular endpoint, DNA damage.

Methods:

- Cells labeled with [^{3}H]Thd
- Irradiations with x-rays at 4˚C, dose rate 3.8 Gy/min
- Irradiations with protons or alphas , horizontal beam, cells at room temp briefly, dose rate = 80-160 Gy/min.

DSB assays

Sucrose gradient sedimentation assay.

- Cells lysed at neutral pH.
- Transferred to top of sucrose gradient.
- Centrifuge 3000 rpm/65 hrs/20˚C.
- Draw fractions from tube bottom through filters that bind DNA.
- Count the filters.

Precipitation assay

- Non-denaturing conditions.
- High salt concentration and cold temp causes high molecular weight DNA to precipitate.
- Centrifuged to separate pellet from supernatant.
- Amount of radioactivity remaining in the supernatant proportional to DSBs.

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Table 1. Sedimentation assay

Radiation	$\alpha \pm$ SE	Ratio $\pm$ SE[a]	RBE $\pm$ SE[b]
p(1)	$0{\cdot}37 \pm 0{\cdot}04$	$0{\cdot}73 \pm 0{\cdot}11$	$0{\cdot}86 \pm 0{\cdot}15$
α(1)	$0{\cdot}51 \pm 0{\cdot}05$		$1{\cdot}19 \pm 0{\cdot}18$
p(2)	$0{\cdot}43 \pm 0{\cdot}04$	$0{\cdot}86 \pm 0{\cdot}11$	$1{\cdot}00 \pm 0{\cdot}15$
α(2)	$0{\cdot}50 \pm 0{\cdot}04$		$1{\cdot}16 \pm 0{\cdot}16$
X-ray	$0{\cdot}43 \pm 0{\cdot}05$		

Linear fits of the data were performed by the least-squares method using $1/M_w = \alpha D + \beta$, with β the common intercept $= (1{\cdot}84 \pm 0{\cdot}23) 10^{-10}$ dalton^{-1} and α in $(10^{11}$ dalton$)^{-1}$ Gy^{-1}.

[a] Ratio for α for protons relative to α for α-particles of the same LET.

[b] Ratio of α value relative to α for X-rays.

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Table 2. Precipitation assay

Radiation	$\alpha \pm SE$	Ratio $\pm$ SE[a]	RBE $\pm$ SE[b]
p(1)	$0{\cdot}14 \pm 0{\cdot}01$	$1{\cdot}08 \pm 0{\cdot}13$	$0{\cdot}74 \pm 0{\cdot}06$
α(1)	$0{\cdot}13 \pm 0{\cdot}02$		$1{\cdot}68 \pm 0{\cdot}09$
p(2)	$0{\cdot}16 \pm 0{\cdot}01$	$1{\cdot}23 \pm 0{\cdot}17$	$0{\cdot}84 \pm 0{\cdot}07$
α(2)	$0{\cdot}13 \pm 0{\cdot}02$		$0{\cdot}68 \pm 0{\cdot}10$
X-ray	$0{\cdot}19 \pm 0{\cdot}01$		

Linear fits of the data were performed by the least-squares method using $Y(D) = \alpha D$, where α is in $10^{-2}\,Gy^{-1}$.

[a] Ratio of α for protons relative to α for α-particles of the same LET.

[b] Ratio of α value relative to α for X-rays.

Results:

- Slopes used to compare the effectiveness of alphas and protons.
- Protons slightly less effective but not statistically significant.
- RBEs compared to X rays are ~ 1 for both protons and alphas in sedimentation assay.
- RBEs for protons and alphas are both **less than 1** in the precipitation assay
- Neither assay can distinguish SSB from DSB

These results are at odds with the results for survival and mutation

Complications?

- Very high doses used.
- Dose rates and irradiation times were significantly different.

Perspective 1992:

- Literature reports on RBE values for DSB induction and yields of DSBs/Gy vary considerably.
- Neither assay can distinguish complex damage or small fragments.