Acute Death of Mice from Massive Doses of High Energy Electrons

Kenji Takeshita
Department of Radiation Biology, Research Institute for Nuclear Medicine and Biology, Hiroshima University

Research Code No: 400
Key Words: LINAC, Dose rate, Electrons, CNS Death, ECG

電子線大線量照射によるマウスの致死効果

広島大学原爆放射能医学研究所障害基礎研究部門
竹　下　健　児

（昭和46年7月28日受付）

35 MeV リニア・アクセラレータによつて加速された 25 MeV 電子線でＩＣＲマウス(雌)に大線量照射を行なつて，その急性致死効果を心電図法により観察した．照射線量率は，パルス繰返し 15 pps で 6.4～13 krad/sec，60 pps で 68 krad/sec，120 pps で 130 krad/sec であつた．

照射中致死までの時間は68krad/sec 以下の照射においてはほぼ線量率に逆比して短縮するが，130 krad/sec でも 2.6 sec を要し68 krad/sec 照射群と差がなかつた．これは外部から加えられた刺激に対して反応を示しうる最少の時間と思われる．少くともマウスの死は 170 pulses の衝撃でもたらされていることになる．

“初期動揺”[1]の現象は全く見られず，照射開始後間もなく激しい痙攣と騒擾が起るが，心電図上で欠搏などが見られるほかは，ほとんど連続的に全身強直性痙攣に移り死に至つた．この痙攣開始までの線量は約19krad であり，^{60}Co γ 線照射[1)7)]で確認された初期動揺終了時の線量18 kRと関連するものと思われる．

フェノバルビタール投与による照射中致死の遷延も 1.9～2.4倍であつて，11 krad/sec 照射群で31 sec，330 krad の結果を得た．

線量率 250～420 rad/pulse で 6～26 pulses 照射の平均生存時間は，線量が 1,600～8,200 rad の範囲であり，予期されたごとく 112～130 hr であつた．

^{60}Co γ 線照射[7)]における照射中致死線量と比べ，15 pps 照射群ではやや大きく，120 pps では比較できなかつた．死亡時期判定は閉回路テレビにより痙攣の最終伸張期としたが，^{60}C oγ 線照射で痙攣開始時期としたものと一致した．高線量率パルスの照射には余分な線量があると思われる．

電子線の線量測定は，Fe$^{\#}$-Cu$^{\#}$ 線量計[2)]を用いて決定した．

With the development of medical accelerators in Japan, acute death of mammals resulting from massive doses of electrons has been studied. Using 60 MeV electrons produced by a linear accelerator of Tohoku University, Sakka et al.[4)] determined mean survival times of mice after irradiation, the

effect of shielding the head during lethal doses, and the difference in behavior of various animals during irradiation.

Concerning the acute death of mice during massive dose irradiation of ^{60}Co gamma-rays after Langham et al.[3], Takeshita et al.[5)7)] reported several results observing electrocardiograms of animals and a pattern of very short duration to death during irradiation was demonstrated. Dose rate effects for death during irradiation were also studied in the range of 10 R/min to 40 kR/min.

The present study of acute effects in mice from massive doses by high energy electrons was observed by the above mentioned technique. A closed circuit television was used to observe the terminal phase of mice, and ferrous-cupric dosimeters were employed to estimate their absorbed doses. Experiments were therefore precisely performed for these very high dose rate exposures.

Method

Electrons were produced by a 35 MeV linear accelerator installed at this institute in 1969, the characteristics of which were previously reported[6]. It is being used for radiobiological studies of exposures similar to that of the Atomic Bomb, and those of radiation therapy with moderate dose rates.

Electrons are accelerated in $2\pi/3$ mode of about 3,000 MHz microwaves with a pulsed duration of 3.2 μsec and a pulse repetition rate of 15, 60 or 120 pps.

In the "research mode", electron energy can be changed continuously between 5 and 39 MeV. The beam current is 320 mA at peak with an average of 90 μA at a nominal operation of 25 MeV. The output of 25 MV X-rays is 14,500 rad/min at an isocenter 100 cm distant from a target. In the "therapy mode", six specific energies are programmed at a range of 5 to 30 MeV by 5 MeV intervals for electrons, and an energy of 8 MeV for X-rays. Dose rates of these radiations are regulated to 300 rad/min in a maximum field of 27×30 cm at the isocenter. The head can rotate through 360°.

During this study, 25 MeV electrons were used in the research mode. Beams were scattered by a 2.0 mm aluminum plate to make the fields as uniform as possible. The head was positioned at 180° Electron doses were measured using ferrous-cupric dosimeters[2], exposed simultaneously with mice under an acrylite plate 1.0 cm in thickness for build-up and on a supporting plate suspended in air at the isocenter.

ICR-JCL female mice of 8 weeks old or more, obtained from Nippon CLEA K.K., were used. To observe time until death during irradiation, mice were individually affixed to electrocardiographic plates as previously described[5], and continuous lead I electrocardiograms were obtained throughout irradiation. Exposure time was checked by a separate timer.

The time of death was taken to be the time of appearance of tonic convulsions electromyographically on each electrocardiogram[5)7)]. In the present study the time of death during irradiation was established precisely, observing the animals on closed circuit television, and it corresponded to the terminal moment with hyperextension after tonic convulsions. The total dose was estimated as the product of the exposure time and the dose rate at that exposure, and was the dose delivered until death.

To control convulsions, 2.5 mg phenobarbital, diluted in ethanol was injected intraperitoneally about 10 minutes before irradiation, as previously described.[7)]

Mean survival times after irradiation were determined for groups of 6 mice each. Exposure was by 1.6 to 8.2 krad corresponding to 6 to 26 pulses of electrons, at various dose rates between 250 and

420 rad per pulse.

Results

Dose rates in the various pulse repetitions are shown in Table 1. Electron beams in the research mode were not so well defined according to series of experiments as shown in this table. Dose rates per pulse for the very short exposure times were not regularly used as shown in Table 4.

Table 1. Output of 25 MeV electrons by pulse repetition rate

Pulse repetition rate (pps)	Dose rate (krad/sec)	
15	6.4 ± 0.8	8.8 ± 0.3
	11 ± 0.9	13 ± 0.5
60	68 ± 1	
120	130 ± 15	

The average values of total doses and times until death during irradiation are shown in Table 2, with their standard deviations. With higher dose rates, the time until death was shorter and the total dose was slightly larger, up to 68 krad/sec. There were no differences in times until death between groups at 68 and 130 krad/sec. Two and eight-tenths or 2.6 sec were necessary to cause death. Mice died following an exposure of at least 170 pulses (60 pps×2.6 sec) of 25 MeV electrons. Doses and times until death following administration of phenobarbital are also shown in Table 2. The effect of anticonvulsives was evaluated by a ratio of total doses until death during irradiation, compared with the untreated group at the nearly same dose rates. Ratios of the total dose in the 11 krad/sec group injected phenobarbital to those in the 8.8 and 13 krad/sec groups were 2.4 and 1.9, respectively.

Table 2. Total dose and time, until death during irradiation

Dose rate (krad/sec)	Number of mice	Time until death during irradiation (sec)	Total dose until death (krad)
8.8	10	16 ± 3	140 ± 30
13	16	13 ± 3	170 ± 40
68	3	2.8 ± 0.3	190 ± 20
130	12	2.6 ± 0.6	340 ± 70
Following phenobarbital administration			
6.4	10	37 ± 3	230 ± 30
11	10	31 ± 3	330 ± 50

Typical patterns of electrocardiograms at each dose rate are shown in Figure 1, with the convulsions immediately after irradiation earlier at higher dose rates. Over 60 pps, signals induced by electrons were often superimposed on them. Arrows indicate the times of death as determined in this study.

As shown in Fig. 1, the phenomenon of initial pitching as described by Fujii[1] was not observed at dose rates from 8.8 to 130 krad/sec. The averages and their standard deviations of times and doses delivered until onset of convulsions after the initiation of irradiation on electrocardiograms are shown in Table 3.

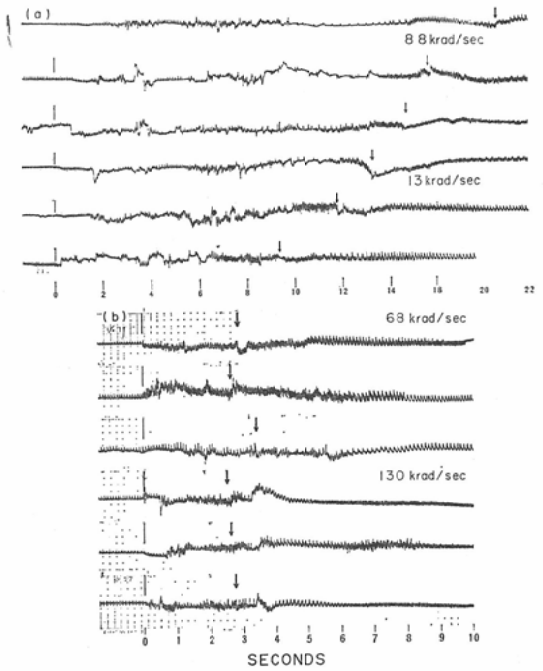

Fig. 1. Patterns on the electrocardiogram until death during irradiation; arrows indicate the time of death in the terminal phase: (a) at dose rates of 8.8 and 13 krad/sec at 15 pps, (b) at dose rates of 68 krad/sec at 60 pps and 130 krad/sec at 120 pps.

Table 3. Comparison of onset of convulsions on electrocardiograms

Dose rate (krad/sec)	Onset time of convulsions (sec)	Total dose until onset (krad)
8.8	2.2 ± 0.6	19 ± 5
13	1.5 ± 0.6	20 ± 7
130	0.3 ± 0.2*	39 ± 35

*Four mice exhibited convulsions on initiating irradiation.

Table 4. Mean survival time after irradiation

Exposed pulses	Dose rate (rad/pulse)	Total dose (rad)	Mean survival time (hr)
6	270	1,600	124 ± 10*
6	290	1,700	130 ± 20*
8	420	3,400	123 ± 17
16	400	6,400	123 ± 11
19	250	4,800	118 ± 4
23	290	6,600	116 ± 4
26	320	8,200	112 ± 11

*Three mice in two groups survived over three weeks.

Mean survival times after irradiation with a few electron pulses are shown in Table 4. Three of 12 mice in two sets of experiments, which received 6 pulses (about 1,600 rad), survived over three weeks. In the other groups up to 8,200 rad, none survived over 130 hours.

Discussion

Takeshita and Fujii[5] have described the instantaneous death of animals following irradiation, with the same object as Langham et al.[3] A dose rate effect was demonstrated in the range of 10 R/min to 40 kR/min of ^{60}Co gamma-rays.[7]

Concerning survival times after irradiation by electrons, Sakka et al.[4] stated that the energy transfer of pulsed radiations to the tissue was lower than those of X- or gamma-rays in their dose estimated from measurements in μC units. It is difficult to calculate the absorbed dose from the charging current because of beam size, the material's thickness and other exposure factors.

Dose estimation for pulsed radiations is also usually difficult with ionization chambers because of recombination of ions produced. In this study, dose was measured by Fe-$^{\#}$Cu$^{\#}$ chemical dosimeters[2], which were responsible for higher dose rates in the very high dose ranges and presumably independent of radiation quality. As shown in Tables 1 and 4, dose rate per pulse varied by exposure, especially for very short exposures of a few pulses.

As shown in Table 2, times of death during irradiation decreased as the dose rate increased, up to 68 krad/sec, and reached a minimum value over that dose rate. The latter suggests that at least 2 seconds were necessary to cause death of animals. For comparison with the result reported previously[7], the relation between the exposure rate and time until death during irradiation is shown in Fig. 2. In this figure the broken line with a gradient of −0.85 was confirmed by Takeshita et al. for ^{60}Co gamma-rays, and data for electrons are in good agreement with this line except for a point at 130 krad/sec (7.8×10^{3} krad/min in Fig. 2). Higher total doses until death during irradiation at dose rates of 68 and 130 krad/sec than those in 15 pps exposure groups are presumably caused by the over-exposure of the higher re-

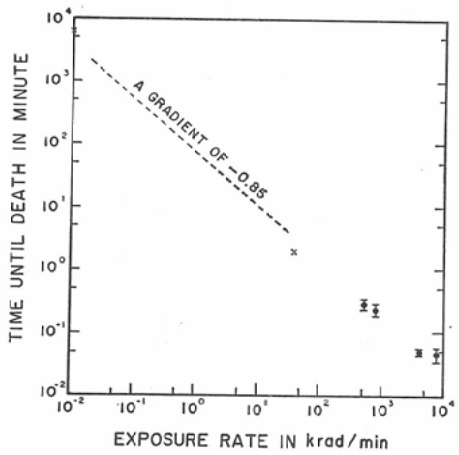

Fig. 2. Relation between the exposure rate and time until death during irradiation. The broken line and "×" marks were obtained from the irradiation by ^{60}Co gamma-rays[7].

petition rate electrons.

One 11 krad/sec group receiving phenobarbital demonstrated an extension of time twice that of the untreated groups, and bore more massive doses of 330 krad.

Observing on electrocardiogram and on television, times of death in the present study were a little later than times of appearance of tonic convulsions; while in previous gamma-irradiations these were nearly the same.

Acute death in Sakka's[4] and our series may be essentially the same between ^{60}Co gamma-rays and pulsed X-rays or electrons, from the point of view that secondary electrons released by these radiations have the main role in radiation damage. The effect that shielding of the head of mice protected from electrons' damage by Sakka et al. is in a good agreement with the result reported in a previous investigation[5] against ^{60}Co gamma-rays. Total doses delivered until death by gamma-irradiation[7] are available for comparison with the total doses for 25 MeV electrons at 15 pps in this study. Ratios are 1/1.3 to 1/2.1 for untreated groups and 1/1.5 to 1/1.7 for groups following phenobarbital administration. A clear wasted portion for electrons exists in both ratios. As one of reasons this may be owing to the lower energy transfer of pulsed radiations.

Initial pitching[1)7)] due to marked movement of short duration after initiating irradiation was not observed in the present study. Exposed mice had some marked movement with convulsions and a few seconds later they were already irreversible. After that reduced heart rates or absence of pulse and an almost continuous transition to tonic convulsions in the terminal stage were observed. Onset of convulsions in about the 10 krad/sec groups was clear and total doses delivered until onset were about 19 krad, while total exposure until the disappearance of initial pitching in gamma-irradiation was 18 kR. At 130 krad/sec, convulsions occurred a little later or just at the start of irradiation, but total dose of 39 krad was not so responsible.

Mean survival times after irradiation by 6 to 26 pulses occurred in the gastrointestinal death range as expected.

Summary

The effects of massive doses of 25 MeV electrons on mice were studied. The maximum dose rate was 130 krad/sec, and the time until death during irradiation was 2.6 sec. Mice died following an exposure of at least 170 pulses. Timing of acute death was delayed following phenobarbital. The mean survival times of mice after irradiation by 6 to 26 pulses were in the range of gastrointestinal death.

Acknowledgements

Our thanks for technical assistance go to Messrs. Sunayashiki, Takeoka and Kato. The chemical dosimetry was performed with the help of Dr. Antoku and Miss Yasuda.

References

1) Fujii, K.: Nipp. Act. Radiol. 25: 30–46, 1965.
2) Hart, E.J.: Radiat. Res. 2: 33–46, 1955.
3) Langham, W., Woodward, K.T., Rothermel, S.M., Harris, P.S., Lushbaugh, C.C. and Storer, J.B.: Radiat. Res. 5: 404–432, 1956.
4) Sakka, M., Sasaki, S. and Inomata, T.: Nipp. Act. Radiol. 30: 566–568, 1970.
5) Takeshita, K. and Fujii, K.: Nipp. Act. Radiol. 23: 40–47, 1963.
6) Takeshita, K., Antoku, S., Sunayashiki, T., Otani, S. and Kazusa, C.: Nipp. Act. Radiol. 30: 687–697, 1970.
7) Takeshita, K., Umayahara, A. and Fujii, K.: Nipp. Act. Radiol. 31: 133–142, 1971.