FROM THE DONNER LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA, USA.

PULSED HIGH-INTENSITY ROENTGEN RAYS

Inactivation of human cells cultured in vitro and limitations on usefulness in radiotherapy

by

PAUL TODD, H. SAUL WINCHELL, JOSÉ M. FEOLA and GARY E. JONES

The suggestion that neoplastic cells are frequently more hypoxic than their normal counterparts, in conjunction with the observation that hypoxic mammalian cells are partially protected from effects of ionizing radiation (DEWEY 1960), has stimulated efforts to seek radiation, the action of which is inpedendent of the presence of oxygen. Some decrease in the 'oxygen effect' with the use of high intensity electron beams has been observed in chemical systems (ROTBLAT & SUTTON 1960), and in bacteria (DEWEY & BOAG 1959), and with high intensity proton beams in water (SHALEK & BONNER 1953). Decreased incidence of chromosome breakage at high radiation dose rates has also been reported (KIRBY-SMITH & DOLPHIN 1958).

If the reduced oxygen effects noted in these experiments with high intensity radiations are to be applicable in clinical radiotherapy, such effects must be present within the appropriate dose range for human treatment. In addition, therapeutic applicability requires the use of radiation with adequate penetration. The recent availability of linear accelerators for the production of high-energy, high-intensity photons suggested experiments to determine the dose dependency of radiation-induced inhibition of colony formation by cultured human cells under aerated and anoxic conditions. Our experimental results define the limitations of such high-intensity radiation in human radiotherapy.

Submitted for publication 8 May 1967.

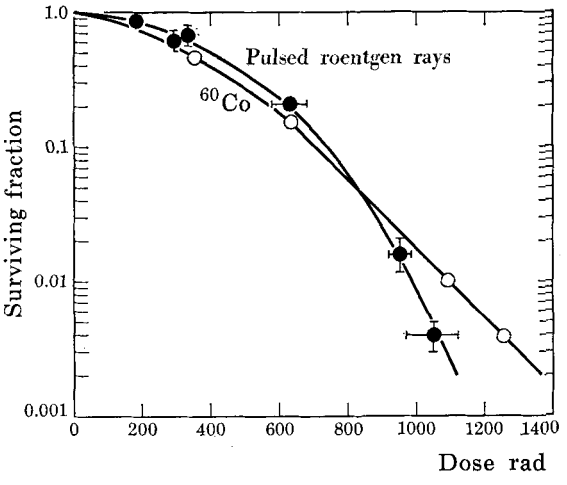

Fig. 1. Survival curves for cultured human kidney cells exposed to ^{60}Co gamma rays at 150 ± 10 rad/min (open circles) and pulsed 10 MV roentgen rays with the total dose being delivered in 3×10^{-8} seconds (solid circles), both under aerated conditions.

Material and Methods. The human kidney cells, line T1 originally obtained from BARENDSEN, used in these experiments, were serially propagated in Eagle's 'Minimum essential medium' and 10 % fetal bovine serum. We irradiated six-to-eight-hour single-cell cultures growing in 35 mm plastic petri dishes on day-old 'feeder layers' of 5×10^4 irradiated (4 000 rad) cells per 35 mm dish.

We used 10 MV roentgen rays generated from a Physics International pulsed electron accelerator in the dose rate range of 10^{12} to 10^{13} rad/min. Each total dose was delivered in a single pulse of 3×10^{-8} seconds duration. For comparison we used ^{60}Co gamma rays at a dose rate of 150 ± 10 rad/min. For both radiations, the dose delivered to each group of exposed cultures was determined by LiF thermoluminescence, a method found to be dose rate independent in the range used. Dosimetry capsules and cultures were simultaneously exposed to each dose. Both radiations were filtered by one inch of polyethylene.

The petri dishes containing the cells to be irradiated were mounted in a mylar film-faced aluminum exposure wheel (TODD 1966). The medium was removed from the petri dishes just prior to irradiation, and the dishes were exposed vertically so that they were perpendicular to the incident radiation beams. In those experiments in which oxygen was removed, high-purity nitrogen was passed over the cultures for 20 min prior to exposure. Subsequent to exposure, the medium was replaced, and the cultures were incubated at 37° C and fed with fresh medium every 5 days until visible colonies developed ($\sim$ 2 weeks). Surviving fractions were determined on the basis of colony counts in exposed and control cultures.

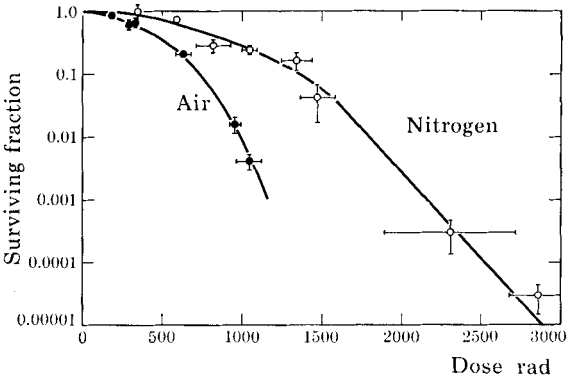

Fig. 2. Survival curves for cultured human kidney cells exposed to pulsed 10 MV roentgen rays. The 'air' curve is the one shown in fig. 1 and the 'nitrogen' curve differs in that purified nitrogen was passed over the cells for 20 minutes prior to irradiation.

Results

Survival curves for cells exposed to pulsed roentgen rays and ^{60}Co gamma rays under aerated conditions are presented in Fig. 1. Vertical error bars represent the standard errors of mean survival propagated from the relative errors of the mean colony counts on control and irradiated plates. Horizontal error bars represent standard deviations of doses determined by the multiple (9 or more) LiF dosimeters used in measuring the dose at each individual dose point. The dose rate in rad/s at each dose of pulsed roentgen rays was different and was equivalent to the dose at that point divided by 3×10^{-8} seconds. The highest dose rate represented in this graph is approximately 3×10^{11} rad/s. The two curves of Fig. 1 do not differ significantly considering the experimental conditions.

The survival curve for cells exposed to pulsed roentgen rays while in a nitrogen atmosphere is presented in Fig. 2 and compared with that obtained under aerated conditions. The results suggest that fully anoxic conditions were not achieved in the experiment, since the ratio of doses in nitrogen to those in air required for equivalent survival is less than that anticipated under complete anoxia. However, it can be concluded that the effect of oxygen at atmospheric pressure did not disappear at these radiation intensities.

Discussion

SHALEK & BONNER, using hydrogen peroxide formation in pure water as their end-point at total proton doses in excess of 10^7 rad, found that the difference between yields in air-saturated water and helium-saturated water disappeared at dose rates greater than 10^8 rad/s. These published observations suggest that all ionization produced at intensities greater than 10^8 rad/s is equally effective in

eliminating the oxygen effect. If the mechanism whereby high intensity reduces the oxygen effect is the 'use-up' of available intracellular oxygen at a rate greater than the diffusion of available oxygen into the irradiated volume, then the minimum dose rate (10^8 rad/s), obtained by SHALEK & BONNER) is a measure of the rate of oxygen diffusion into the irradiated volume.

DEWEY & BOAG, using dose rates in excess of 10^8 rad/s (actually about 10^{10} rad/s), found that doses greater than 13 000 rad were adequate for eliminating the effect of 1 % oxygen in nitrogen in irradiated cultures of the bacterium *Serratia marcescens*. If we take the value of 13 000 rad as representing the minimum dose required for the 'use-up' of intracellular oxygen at this partial pressure (about 7.6 mm Hg), then one would anticipate a required dose of 260 000 rad ($20 \times 13\,000$) to 'use-up' the intracellular oxygen at atmospheric pressure (about 152 mm Hg). Recently, EPP, WEISS & FENLON (1967) determined that the threshold for oxygen use-up in bacterial strain *E. coli* B/r in air is about 60 000 rad.

It is therefore not surprising that the experiments we describe in this work did not demonstrate a reduced oxygen effect under aerated conditions at dose rates of 10^{11} to 10^{12} rad/s and in the dose range of 200 to 1 050 rad.

On the basis of the quantitative considerations just presented, an estimation may be made of the required partial pressure of oxygen in tissue to allow complete oxygen 'use-up' by therapeutically usable radiation doses. If we assume 2 000 rad to be the largest single acceptable therapeutic dose, then a high-intensity radiation pulse (delivered at more than 10^8 rad/s) of this magnitude would require that the oxygen partial pressure in the irradiated tissue be less than 1.1 mm Hg to insure 'use-up' of the residual oxygen with resultant elimination of the oxygen effect. Therefore, in order to utilize in radiotherapy the diminished oxygen effect seen in high-intensity radiation experiments, it would be necessary to reduce the partial pressure of oxygen in the target-area tissue to about 1.1 mm Hg for the duration of the radiation pulse. Such a procedure is limited by considerations of the human physiologic effects of temporary extreme hypoxia.

Acknowledgements

We are greatly indebted to Mrs C. L. Boerke and Mrs W. M. Jackson for unstinting laboratory assistance and to Mr B. Bernstein and the staff of Physics International, San Leandro, California, for performing the irradiations and dosimetry, and for their generous sharing of their facilities and equipment. This work was supported by NASA and AEC.

SUMMARY

Pulsed 10 MV roentgen rays administered at dose rates of 10^{11} to 10^{12} rad/s and in the total dose range of 200 to 1 050 rad/pulse did not differ significantly from ^{60}Co gamma rays in their ability to inactivate aerated cultured human cells. It is estimated that a total dose

of 260 000 rad delivered at dose rates greater than 10^8 rad/s is necessary to eliminate the oxygen effect in aerated cells and that at dose rates greater than 10^8 rad/s the partial pressure of oxygen in tissue would have to be reduced to less than about 1.1 mm Hg prior to irradiation to eliminate the oxygen effect.

ZUSAMMENFASSUNG

Pulsierende 10 MV Röntgenstrahlen bei Dosisverhältnissen von 10^{11} bis 10^{12} rad/s, und bei Dosen von 200 bis 1 050 rad/Puls waren nicht signifikant verschieden von ^{60}Co Gammastrahlen für die Inaktivierung von Menschenzellen in aerobischer Kultur. Es wurde berechnet, dass man, um den 'Sauerstoffeffekt' bei Dosisverhältnissen grösser als 10^8 rad/s zu beseitigen, mindestens 260 000 rad benötigt. Für die Beseitigung des Sauerstoffeffekts bei Dosisverhältnissen grösser als 10^8 rad/s, muss der Sauerstoffdruck weniger als 1.1 mm Hg sein.

RÉSUMÉ

Les rayons de roentgen pulsés de 10 MV administrés avec un débit de dose de 10^{11} à 10^{12} rad/s et à des doses totales allant de 200 à 1 050 rad/impulsion ne présentent pas de différence significative avec les rayons gamma du ^{60}Co en ce qui concerne l'inactivation de cellules humaines en cultures aérées. Les auteurs estiment qu'il faut une dose totale de 260 000 rad administrés avec un débit supérieur à 10^8 rad/s pour supprimer l'effet oxygène dans les cellules aérées et que pour des débits de dose supérieurs à 10^8 rad/s, il faudrait réduire la pression partielle d'oxygène dans le tissu à moins de 1,1 mm de Hg environ, avant l'irradiation, pour éliminer l'effet oxygène.

REFERENCES

BARENDSEN G. W., BEUSKER T. L. J., VERGROESEN A. J. and BUDKE L.: Effects of different ionizing radiations on human cells in tissue culture. II. Biological experiments. Radiat. Res. 13 (1960), 841.

DEWEY D. L.: Effect of oxygen and nitric oxide on the radiosensitivity of human cells in tissue culture. Nature 186 (1960), 780.

— and BOAG J. W.: Modification of the oxygen effect when bacteria are given large pulses of radiation. Nature 183 (1959), 1450.

EAGLE H.: Amino acid metabolism in mammalian cell cultures. Science 130 (1954), 432.

EPP E. R., WEISS H. and FENLON A.: Observations of the oxygen effect and radiation recovery in bacterial cells irradiated with high-intensity pulsed electrons. Radiation Res. 31 (1967), 646.

KIRBY-SMITH J. S. and DOLPHIN G. W.: Chromosome breakage at high radiation dose rates. Nature 182 (1958), 270.

ROTBLAT J. and SUTTON H. C.: The effects of high dose rate of ionizing radiations on solutions of iron and cerium salts. Proc. roy. Soc. A 253 (1960), 490.

SHALEK R. J. and BONNER T. W.: Formation of hydrogen peroxide in water by 1-MeV protons. Nature 172 (1953), 259.

TODD P. W.: Reversible and irreversible effects of densely ionizing radiations upon the reproductive capacity of cultured human cells. Med. Coll. Virginia Quart. 1 (1966), 2.