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Quantitative analysis of the response of epidermis to two-dose irradiations

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1. Introduction

Promising data have been obtained on the effects of non-standard fractionation schemes for the treatment of some types of tumours; the results show that some tissues and tumours are more radiosensitive when unconventional courses of fractionation are used (Sakamoto and Phillips 1979). This fact would indicate that some effects can be exerted by increasing or decreasing the time between fractions. The *in vivo* investigation of the intimate processes that occur in cells after split irradiations seems to be relevant.

Epidermal cells react after irradiation, demonstrating a definite and measurable volume increase. This parameter, which has been characterized as dose-dependent and post-irradiation-time dependent (Lanfranchi *et al.* 1979, Klein Szanto *et al.* 1977) is related to the hyperactive state reached by epidermal cells during the acute phase of radiation injury (de Rey *et al.* 1979). Basal cell volume is a relatively easy end-point to measure and a reliable tool to express the *in vivo* effects using tissue sections. It also provides good evidence of the cellular behaviour after fractionated irradiations.

In the present publication we have reported the results obtained measuring volume variations in basal cells *in vivo* after irradiation with single or two-dose irradiations, varying the time interval between the two fractions.

2. Materials and methods

The distal halves of the tails of 5-day-old Wistar rats were irradiated in separate trials with 10, 20, 40, 80 and 160 Gy X-rays with a Philips 250 Radiotherapy machine at 250 kV and 13 mA with a 0·25 mm Cu + 1 mm Al filter, at a dose rate of 5 Gray/min. The proximal portion of the tail of the animals was used as control and it remained shielded together with the animal body. The distal half of the tails were submitted to single doses or fractionated doses (split in two equal fractions). The time intervals between fractions were 10, 30, 60 and 120 min and 24 hours. Five animals were sacrificed for each condition 4 days after the onset of the irradiations which, according to previous experiments, is the time point in which maximum effects were noted (Lanfranchi *et al.* 1979, Klein Szanto *et al.* 1977, de Rey *et al.* 1979).

Skin samples obtained from tails were processed for electron microscopy: small pieces of whole skin were cut perpendicular to the axis of the tail and fixed with 2 per cent osmium tetroxide. They were later dehydrated and flat-embedded in Maraglas. Thin sections were obtained.

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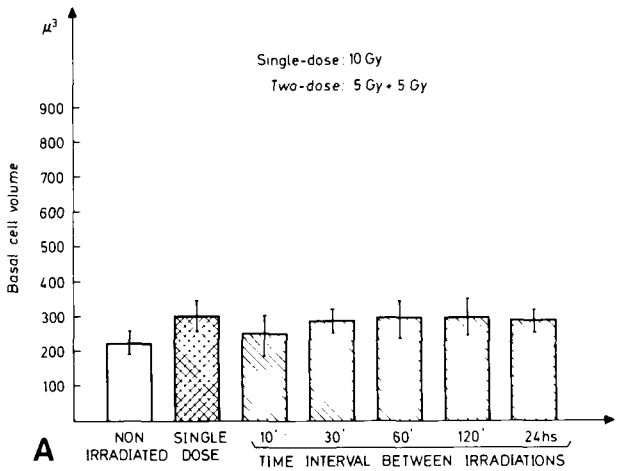
Absolute values for cell volumes (V) and the numerical density of basal keratinocytes (N_v) were determined by stereologic calculations. These procedures have been extensively described in previous publications (Klein Szanto *et al.* 1977). Briefly, the (N_v) numerical density was determined by the equation

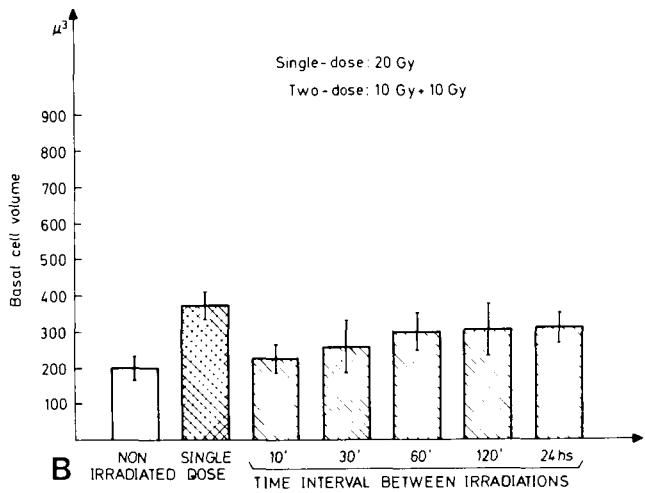
$$N_v = N_a^{\frac{3}{2}} / (V_v^{\frac{1}{2}} \beta)$$

where β is a coefficient related to the cell shape (in this case 1·6). N_a is the number of cells per unit area and V_v is the volume density of the structure under study. The absolute volume of the epithelial cells was calculated using the following

$$V \text{ cell} = \frac{10^9}{N_v \text{ layer}} \mu m^3$$

Four hundred basal cells were measured for each irradiation condition (single-dose or two-dose). Two hundred basal cells were scored for non-irradiated cases. They





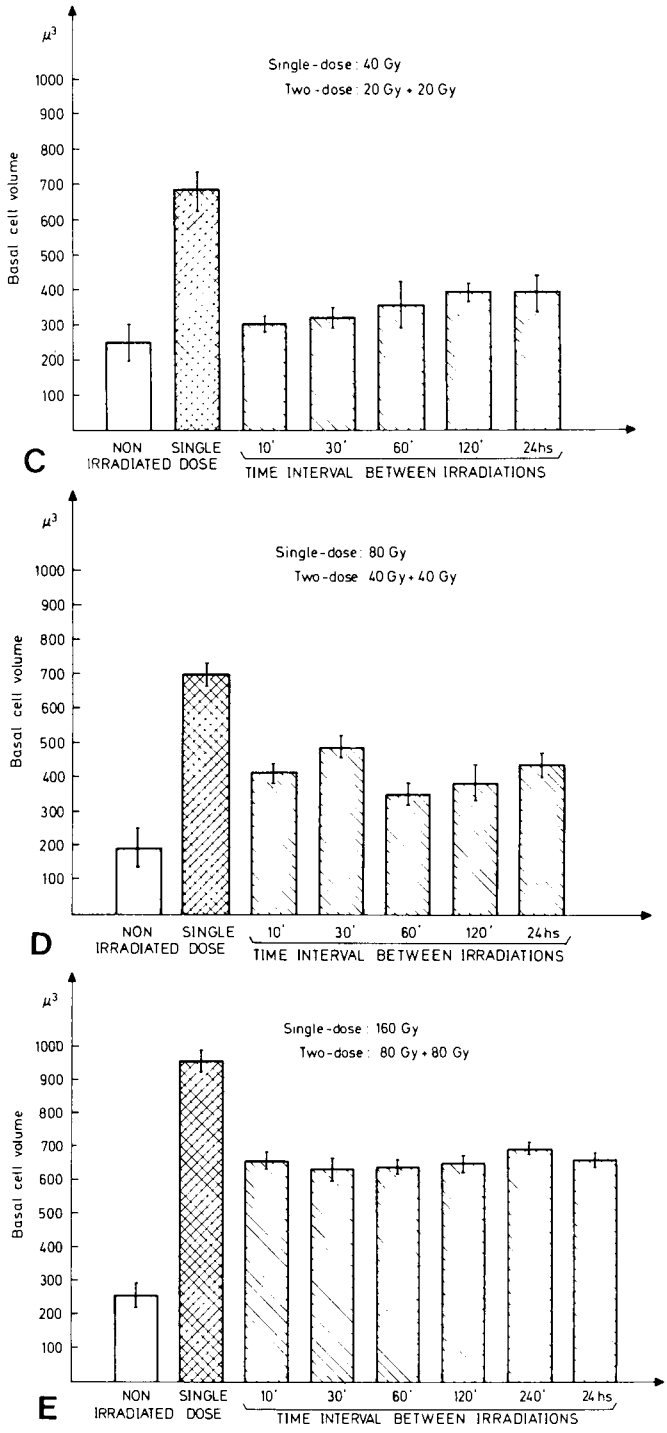


Figure 1. Basal cell volumes reached after single- or two-dose irradiations are compared. A: 10 Gy (single dose), 5 Gy + 5 Gy (two doses separated by increasing time intervals); B: 20 Gy (single dose) 10 + 10 Gy (two doses separated by increasing time intervals); C: 40 Gy (single dose), 20 Gy + 20 Gy (two doses separated by increasing time intervals); D: 80 Gy (single dose), 40 Gy + 40 Gy (two doses separated by increasing time intervals); E: 160 Gy (single dose), 80 Gy plus 80 Gy (two doses separated by increasing time intervals).

correspond approximately to 1·5 mm and 0·7 mm of epithelium of irradiated and non-irradiated animals, respectively.

Stereological and statistical calculations were performed with the aid of a Hewlett Packard Calculator 9810.

3. Results

Significant volume increases of basal cells measured *in vivo* 4 days after irradiation with 10, 20, 40, 80 and 160 Gy are represented in figure 1. Cell volume values attained after single exposures are compared with results obtained after two-fraction exposures.

A definite increase in absolute cell volume was observed in all experimental conditions. When the irradiations were delivered in two equal fractions, the volumes reached by basal cells after 20, 40, 80 and 160 Gy were 26, 43, 45 and 32 per cent respectively, lower than the volumes reached after single exposures. Furthermore, irrespective of the time elapsed between fractions (10 min up to 24 hours) for a given total dose, the values obtained after split irradiations were similar.

Except for the data corresponding to 40 Gy, similar volumes were attained by cells of the basal layer when the tissue was irradiated with single doses or when the dose delivered was twice the single dose but divided in two equal fractions (figure 2).

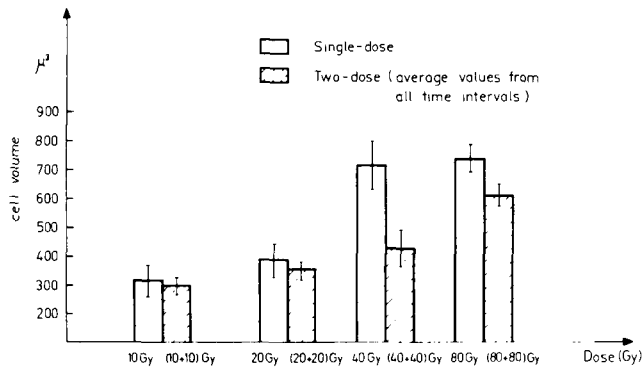


Figure 2. Basal cell volumes obtained after irradiating the epidermis with single doses are compared with basal cell volumes reached after irradiating with twice the single dose but divided into two equal fractions. (Average values from all the time intervals analysed have been plotted.)

4. Discussion

The acute response of epidermal cells to single or fractionated irradiations has been analysed *in vivo* by means of morphometric techniques. A close relationship between volume increase and dose has been described for single doses (Lanfranchi *et al.* 1979, Klein Szanto *et al.* 1977).

A correlation between cell volume increase and several complementary data have been analysed in order to understand better the cell reactions to irradiation (de Rey *et al.* 1979). These studies have revealed cellular metabolic alterations which in most cases resulted in augmented metabolic activity.

As was expected, fractionating the dose in two equal fractions induced lower volume values than non-fractionated equivalent single doses. Several authors have demonstrated similar results analysing other effects in cells *in vitro* (Denekamp *et al.*

1966) and *in vivo* (Withers and Elkind 1969). However, a striking result was that the cell response was not affected by shortening the lapse between fractions. Lapses as short as 10 min were enough to allow recovery of the tissues, but no differences in volume values were detected as function of the time between fractions up to 24 hours. These data together with those shown in figure 2, in which no effect was demonstrated to be exerted by the second dose in fractionated trials, revealed an immediate reaction after the first dose, suggesting an enormous capability of repair within 10 min after irradiation. Neither the second dose nor the different length of the intervals between doses were able to modify this 'fixed' effect of the first dose.

Although the biological mechanisms involved in this unique response to two-dose irradiation are largely unknown, several hypotheses might be outlined.

The ineffectiveness of the second dose could be partly due to the presence of large numbers of cells that would have been rendered hypoxic by the first radiation fraction and consequently more radioresistant. It was shown that from 24 hours onwards the epithelial cells change the oxidative pathway of the Krebs cycle towards the pentose shunt which requires less oxygen tension (Cabrini *et al.* 1970). However, more information is needed regarding oxidative metabolic changes, particularly at shorter post-irradiation times, in order to ascribe radioresistance to intracellular hypoxia.

Cell cycle progression to more radioresistant phases (G_2) could also be suggested as a possible explanation, but only for the longest intervals between doses (Young and Fowler 1969).

Although repair in skin cannot be attributed to systemic release of chemicals (Denekamp and Fowler 1966), the rapid and prolonged reaction herein described could be due to the release of locally acting substances. The existence of some mechanism and/or substances that conditioned the cell to remain unaffected to the second dose was proposed by Powers (1961). This author suggested the existence of a reparative substance pool that becomes exhausted when the first dose is given.

Considering that cell volume changes indicate that both short and prolonged intervals have a similar effect on keratinocyte repair mechanisms, this approach could be suitable to determine the effect of experimental fractionation schemes, especially those involving epithelial tumour treatments. Furthermore this rapid repair phenomenon might be of importance in clinical radiation therapy when rapidly growing tumours are considered.

Summarizing, this report shows a dose-dependent change in epidermal basal cell volume (volume increasing with dose), a reduction in volume change if the dose is split in two fractions, a lack of dependence of this change in volume with fractionation on the time between doses (10 min to 24 hours). When the doses are split the effect is approximately half that of a single dose.

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