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Letter to the Editor

Low Radioprotection by Thiol in Lung: the Role of Local Tissue Oxygenation

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THE VARIATION in radioprotection in different mouse tissues for equivalent doses of the radioprotector drug WR-2721 creates a problem in the application of this compound in clinical practice. The protection factor† ranges from 1.2 in lung and kidney to 3.0 in bone marrow [1–3]. Even within one tissue, lung, we have observed significantly greater protection for the late fibrotic phase of damage than for the early pneumonitis [4, 5]. The factors determining the magnitude of protection are likely to be several, including size of X-ray dose, levels of endogenous and exogenous sulphhydryl in the tissue and the local intracellular concentration of oxygen [6, 7].

The epidermal clone assay has been used to demonstrate that WR-2721 radioprotection varies over the range 1.0–2.2 if the oxygen concentration in the gas surrounding the mice is varied between 0 and 100% [8, 9]. These data revealed that the maximum protection is seen when mice breathe air or 50% oxygen, and the protection falls in both hypoxic and hyperoxic conditions. The data also permitted the oxygen concentration to be determined which allowed half the full oxygen sensitization to be achieved relative to mice irradiated in nitrogen. This 'K_{insp}' value was measured as 10% oxygen for mouse skin. The maximum protection was therefore seen just above the 'K_{insp}' value, as would be expected if oxygen and sulphhydryls are competing for fixation and repair of radiation induced radicals [9]. A similar relationship has been reported *in vitro* with bacteria and with mammalian cells. The addition of cysteine to *Serratia marcescens* resulted in a shift in the oxygen K curve from 0.1 to 10% oxygen [10]. Lunec [11] showed that the maximum radioprotection with dithioerythritol occurred with 0.3% oxygen (cf. 0 and 21% oxygen), which is again close to the K value for those cells. These two *in vitro* systems have K values that are 1–2 orders of magnitude below the level of oxygen in the gas surrounding the mice in the skin clone experiments. Although no direct information is available for the oxygen concentration in the critical cells in the skin when the mice breathe different oxygen concentrations, it has been shown that direct diffusion from the surrounding gas is important [12].

It seemed possible that some of the observed tissue-to-tissue variability in radioprotection could result from variations in intracellular levels of oxygen. Therefore we have carried out a pilot experiment in which mouse lungs have been irradiated with and without WR-2721 whilst the mice were surrounded by gases containing oxygen concentrations of from 7 to 100%. Lower concentrations of oxygen are also of interest, but this will require the development of a high-dose-rate irradiation set up with electrons because the animals do not survive the necessarily prolonged exposure to lower oxygen concentrations during X-irradiation. The lung was chosen for study because it was anticipated that it might be relatively hyperoxic, with the cellular oxygen level being close to that in the alveolar sacs and hence easily modifiable by modifying the inspired gas.

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†Dose with protector divided by dose without protector to cause the same biological effect.

The whole thorax of CBA male mice was irradiated with 240 kVp X-rays (HVL 1.3 mm Cu) without anaesthetic, in the jigs designed by Travis *et al.* [13]. The dose rate was 180 rad/min, with exposure times ranging from 6 to 16 min. The jig assembly was modified to accept a Perspex cover, which was used for the irradiations with the special mixtures of gas. The mice were exposed to the test mixture for 30–60 sec at a high flow rate before irradiation started [14].

Each schedule was designed to give a pair of dose–response curves, for mice irradiated with or without 400 mg/kg WR-2721. Four to eight dose points with 4–6 mice in each dose group were irradiated for each curve (total 296 mice). WR-2721 was administered by i.p. injection 30–40 min before irradiation as a fresh solution made up in distilled water. After irradiation the mice were tested using a breathing rate assay in a whole-body plethysmograph [15] at monthly intervals beyond 12 weeks.

Figure 1 shows the raw data for breathing rates plotted as a function of X-ray dose at 28 weeks after irradiation. Each panel represents a different gas mixture. Sham-irradiated mice had average breathing rates of 336 ± 4 BPM. Dose–response curves were obtained for all 12 schedules, although in some of them many mice had been lost due to excessive pulmonary damage and only two dose groups survived in some of the schedules. These data have been used to calculate protection factors by comparing the dose needed to give the same breathing rate increase, i.e. at 370 breaths/min. These PF values increase progressively from 1.26 in 100% oxygen to 1.56 in 7% oxygen.

The data have also been analysed at the later time of 40 weeks when increased breathing rate and lethality itself have been used as the end point. The three analyses are presented in Table 1. All these assessments indicate a progressive increase in the protection factor with decreasing oxygen tension, but the maximum PF is still lower than that for skin clones or bone marrow [3, 9]. An average of the three values is shown in the final column.

Denekamp *et al.* [8, 9] showed that the dose to give a constant level of clones surviving (e.g. 50%) could be used as a parameter for skin radiosensitivity in varying oxygen concentrations. Their data for skin irradiated with X-rays alone or in the presence of 400 mg/kg WR-2721 are reproduced in Fig. 2 as thin lines. The response of skin in the presence of WR-2721 can be interpreted as a shift in the oxygen 'K_{insp} curve' to even higher levels than the 10% observed in the absence of the drug. The data points and the heavy lines represent the results from these lung experiments. The doses to produce a specified breathing rate or lethality (at 40 weeks) have been

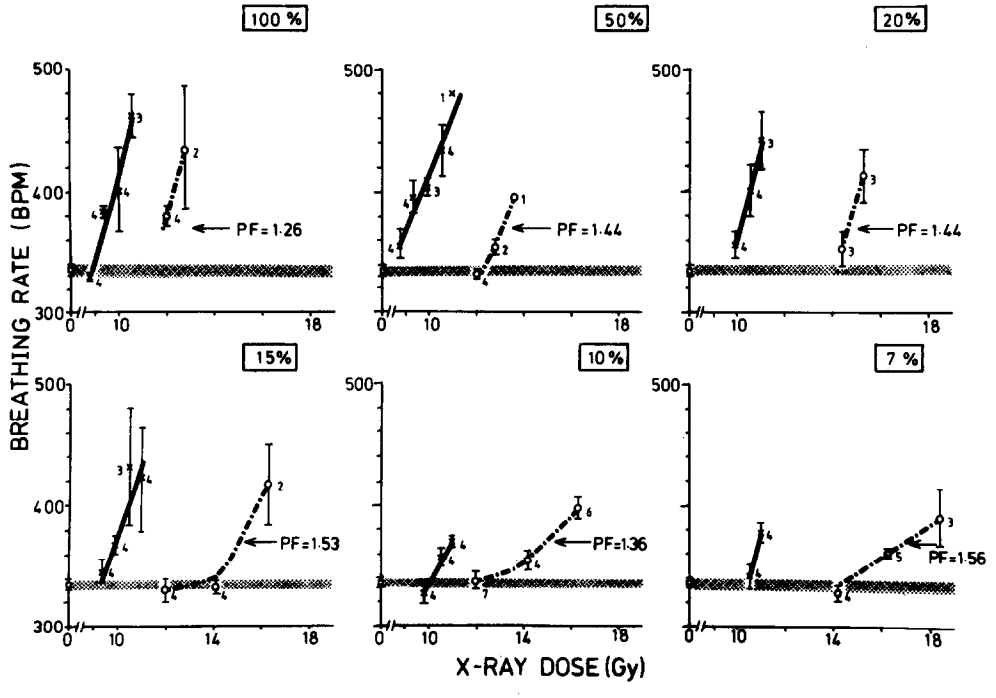

Fig. 1. Breathing rates measured at 28 weeks *after irradiation with X-rays alone or combined with* 400 mg/kg *WR-2721. The mice were breathing the stated oxygen concentrations during irradiation. Changing the gas mixture from* 100 *to* 7% *oxygen had little effect on the mice treated with X-rays alone, but there was a marked increase in the protection afforded by the aminothiol. The vertical bars are* ± 1 *S.E.M. X = X-rays alone,* 0 = *X* + *WR-2721. Nos of mice are indicated at each point.*

Table 1. *Protection factors for* 400 mg/kg *WR*-2721

[O_2] inspired	Breathing rate 370 BPM 28 weeks	40 weeks	Lethality (LD_{20}) 40 weeks	Average
100	1.26 ± 0.06	1.32 ± 0.07	1.28 ± 0.05	1.29
50	1.44 ± 0.05	1.39 ± 0.06	1.35 ± 0.04	1.39
20	1.44 ± 0.04	1.45 ± 0.07	1.41 ± 0.06	1.43
15	1.53 ± 0.10	1.51 ± 0.06	1.49 ± 0.13	1.51
10	1.36 ± 0.04	1.60 ± 0.08	1.57 ± 0.15	1.51
7	1.56 ± 0.12	1.72 ± 0.06	—	1.64

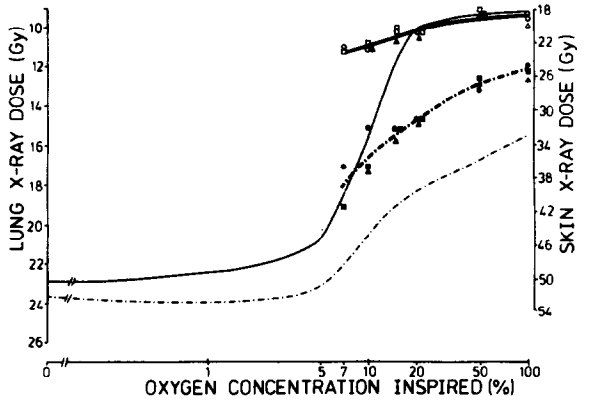

Fig. 2. *Radiosensitivity of mouse lung and mouse skin as a function of inspired oxygen concentration for mice treated with X-rays alone (open symbols) or after* 400 mg/kg *WR*-2721 *(closed symbols). Note reversed vertical dose scales. Thin lines represent epidermal clone data from* [8, 9]. *Thicker lines with points represent present lung data.* ●○ 370 *BPM at* 28 weeks; ■□ 370 *BPM at* 40 weeks. ▲△ LD_{20} *at* 40 weeks.

plotted against the oxygen concentration inspired. The dose scales have been set with a similar ratio of 2.7 (i.e. corresponding to the average OER for skin).

The data for lungs irradiated in the absence of WR-2721 show only a slight change in radiosensitivity over the range of 100–7% oxygen. By contrast, the data for mice irradiated with WR-2721 show a marked increase in radioresistance as the oxygen concentration falls. This is interpreted as a shift in the 'K_{insp} curve' for lung in a manner similar to that for skin. Because the low X-ray dose rate prevented exposure of the lungs to lower oxygen levels, the 'K_{insp}' value could not be determined. Further experiments are planned to extend these lung data to lower oxygen levels using high-dose-rate electrons.

The data presented support the hypothesis that the low radioprotection observed in lung results from the more hyperoxic state of this tissue than of skin in air-breathing mice. The effectiveness of WR-2721 can be increased simply by decreasing the inspired oxygen concentration. The protection factor for late radiation fibrosis was significantly higher (1.45 at 40 weeks) than that for early pneumonitis (1.29 at 20 weeks), as reported previously [4, 5]. It could be argued as evidence for two different sites of injury in the lung, with the tissue or cell type leading to late fibrosis being less well oxygenated than the tissue or cell type leading to early pneumonitis. However, further studies are needed to elucidate the other factors that are also believed to be important, i.e. endogenous SH levels, concentrating capacity for WR-2721 and the level of X-ray dose per fraction. These studies are in progress.

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