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A Comparison of the Radiation Response of the Epidermis in Two Strains of Pig

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The response of the epidermis was compared in two strains of pig, the English Large White and the Göttinger Miniature, after irradiation with ^{90}Sr β rays. The effects of two types of anesthesia were also tested in pigs of each strain, a volatile gas mixture of ~70% oxygen, ~30% nitrous oxide, and 2% halothane, and an intravenously administered narcotic azaperon/etimodat with the animals breathing air. Strain- and anesthetic-related changes were compared on the basis of dose–effect curves for the incidence of moist desquamation from which ED_{50} values (±SE) were determined, i.e., the dose required to produce this effect in 50% of the fields irradiated. For English Large White pigs anesthetized with the volatile gas mixture, an ED_{50} of 27.32 ± 0.52 Gy was obtained for moist desquamation. Irradiation with the azaperon/etomidat anesthesia in this strain of pig produced a significantly higher ED_{50} of 33.36 ± 0.76 Gy ($P < 0.001$). This appeared to be related to the fact that the animals were breathing air, i.e., a lower oxygen concentration (~21%), at the time of irradiation. For the Göttinger Miniature pig the ED_{50} values for moist desquamation were 38.93 ± 3.12 Gy and 43.36 ± 1.34 Gy while using the gaseous anesthetic mixture and the azaperon/etomidat anesthesia with the animals breathing air, respectively. These ED_{50} values are 10–11 Gy higher than those obtained for the English Large White pig under identical conditions of anesthesia, which resulted in a strain difference ratio of ~1.35. Radiation under the volatile gas mixture anesthesia resulted in a uniform irradiation response over the skin of the flank in both strains of pig. Radiation under azaperon/etomidat anesthesia resulted in a nonuniform skin response over the flank. The ED_{50} for moist desquamation was significantly higher in dorsal sites on the flank compared with the ventral area of English Large White pigs; a similar trend was seen in Göttinger Miniature pigs. This difference in the radiosensitivity over the flank skin while the animals are under azaperon/etomidat anesthesia at the time of irradiation was associated with the animals breathing air and is in agreement with findings published previously for animals under halothane anesthesia and breathing air. © 1990 Academic Press, Inc.

INTRODUCTION

The responses of normal tissues to radiation are to a great extent influenced by their structure, function, and cell kinetic organization. Knowledge of the mechanisms involved in these radiobiological responses is often limited, although a comparative study of tissue reactions in different strains of animals can be helpful in obtaining a better understanding of the underlying mechanisms. Such comparative studies have been carried out for the lung (*1*) and testis (*2*) of different strains of mouse, and for the heart of rats (*3*).

In a recent publication a comparison was made between the data on cell kinetics for the epidermis of the English Large White pig and the Yorkshire pig (*4*). These data suggest that differences in radiosensitivity between these two strains of pig and the time of onset of moist desquamation could be related to differences in the normal turnover rate of their respective epidermis. In this paper the results of a study, which formed part of the collaborative programme of the European Late Effect Project Group (EULEP), are being presented. Differences in the radiosensitivity of the epidermis of English Large White (Oxford) and Göttinger Miniature pigs (Neuherberg) are compared. Changes in the latency and incidence of moist desquamation after irradiation with β rays were used as end points. Different methods of anesthesia had also been in standard use in the two laboratories, and hence their effect on the radiation response of the epidermis in the two strains of pig was also investigated. The irradiation of the epidermis with β rays and the evaluation of the epidermal response are well-established procedures and provide highly reproducible results (*5, 6*).

MATERIALS AND METHODS

Female pigs of the English Large White strain were used for studies at the Research Institute, Churchill Hospital, Oxford. These pigs were ~12 weeks of age and weighed ~25 kg at the time of the start of the studies. The irradiation procedures, the methods used to assess the skin damage, and the analysis of the results have been described in detail elsewhere (*5*). Briefly, prior to irradiation the hair on the flanks of the pigs was clipped and 16 fields on each flank, 22.5 mm in diameter, were marked out by tattooing with India ink. These fields were irradiated with single doses of β rays from ^{90}Sr plaques. Details of the dosimetry method and depth doses for the β emitter have been published previously (*7, 8*).

In the laboratories of the GSF, Neuherberg, miniature female pigs of the Göttinger strain were used. These animals were mature at the time of irradiation and weighed ~35 kg. The procedures for marking out the skin fields on each flank and for irradiation were the same as those described for

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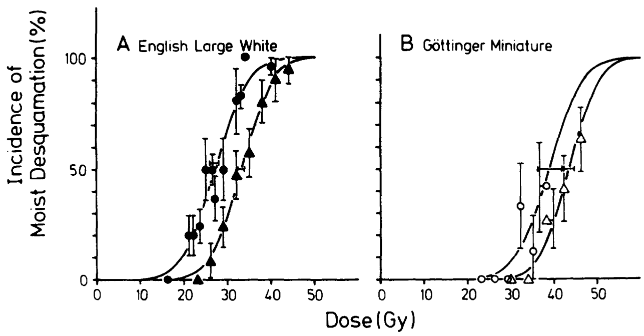

FIG. 1. Dose-related changes in the incidence of moist desquamation after the irradiation of the skin with single doses of β rays from ^{90}Sr plaques on English Large White (A) and Göttinger Miniature pigs (B). Two types of anesthesia were used, a gas mixture of ~70% oxygen, ~30% nitrous oxide, and 2% halothane (●, ○), and an intravenously infused narcotic azaperon/etomidat with the animals breathing air (▲, △).

English Large White pigs. The skin fields of both the English Large White and Göttinger Miniature pigs were irradiated with the same ^{90}Sr plaques.

In the initial studies English Large White pigs and Göttinger Miniature pigs were anesthetized during irradiation with a gas mixture of 2% halothane, ~70% oxygen, and ~30% nitrous oxide (*9*). In a second series of investigations English Large White pigs were premedicated by the intramuscular injection of a neuroleptical compound azaperon (up to 10 mg/kg body weight) in combination with atropin (0.2 mg/kg). This was followed by a single intravenous injection of 1.2–1.8 mg/kg hypnomidate (etomidat), which acted as a hypnotic (*10*). Over the period of irradiation the pigs were maintained under anesthesia by the intravenous infusion of ~2 mg/kg hypnomidate in a 5% glucose solution per hour. The Göttinger Miniature pigs received 1.5–2.5 mg/kg azaperon in combination with atropin (0.2 mg/kg) injected intramuscularly as a premedication. This was followed by a single intravenous injection of 0.4–0.5 mg/kg hypnomidate. Over the period of irradiation anesthesia was again maintained by the intravenous infusion of ~1 mg/kg hypnomidate in a 5% glucose solution per hour.

In both laboratories the radiation responses of the skin were assessed weekly for 9 weeks after irradiation by at least three observers. The skin was scored for the presence of erythema, dry and moist desquamation. The incidence of moist desquamation was used as an end point, and based on these data quantal dose–response curves were constructed by probit analysis (*5*). The ED_{50} values (±SE) for moist desquamation were determined from these dose–effect curves, i.e. the dose of radiation required to produce moist desquamation in 50% of the skin fields irradiated. The effects of the different experimental protocols in the two strains of pig were compared on the basis of these ED_{50} values.

RESULTS

The dose–effect curves showing the incidence of moist desquamation in the English Large White pig after irradiation using both types of anesthesia are given in Fig. 1a; similar curves for the Göttinger Miniature pigs are presented in Fig. 1b. The ED_{50} values (±SE) for moist desquamation obtained from these curves are summarized in Table I. With an anesthetic gas mixture of 2% halothane, ~70% oxygen, and ~30% nitrous oxide an ED_{50} value of 27.32 ± 0.52 Gy was observed for the English Large White pig. Using azaperon/etomidat as the anesthetic and with the pigs breathing air, a significantly higher ED_{50} of 33.36 ± 0.76 Gy was found ($P < 0.001$).

In the Göttinger Miniature pig the ED_{50} value for moist desquamation was 38.93 ± 3.12 Gy for animals anesthetized with 2% halothane, ~70% oxygen, and ~30% nitrous oxide. A relatively large standard error (~8%) was associated with the ED_{50} value since only a small number of skin fields, four to six per dose level, were available for analysis. When the Göttinger Miniature pig was anesthetized using azaperon/etomidat and breathing air, an ED_{50} value of 43.36 ± 1.34 Gy was obtained. This value was not significantly different from that of 38.93 Gy for miniature pigs irradiated using the gaseous anesthesia ($0.1 < P < 0.2$).

The ED_{50} values obtained for moist desquamation in the Göttinger Miniature pig were significantly higher ($P < 0.001$) than those obtained for the English Large White pig. The differences in ED_{50} indicated strain difference ratios of 1.42 ± 0.12 and 1.32 ± 0.06 for the gaseous and intravenously administered anesthetic, respectively (Table I). These strain difference ratios were not significantly different, suggesting an average ratio of ~1.35.

The results obtained for the two strains of pig, under different conditions of anesthesia, were subsequently subdivided according to the position of the fields on the flank. The results of this analysis are presented in Table II. The Large White pig showed no significant variation in ED_{50} value with field position when a gas mixture of halothane, oxygen, and nitrous oxide was used. Irradiation of the English Large White pig under azaperon/etomidat anesthesia resulted in an ED_{50} value of 36.28 ± 1.13 Gy for dorsal-positioned skin fields, which was significantly higher ($P < 0.001$) than that of 30.44 ± 1.26 Gy for fields on the ventral site. In the Göttinger Miniature pigs a similar trend was observed; when the gas mixture was used for anesthesia no significant variation was found in the ED_{50} values for the

TABLE I

Variation in ED_{50} (±SE) Values (Gy) for Moist Desquamation and Their Ratio for Two Strains of Pig Using Different Methods of Anesthesia for Irradiation with ^{90}Sr β Rays

Anesthetic	Strain: English Large White	Strain: Göttinger Miniature	Ratio (±SE)
~70% oxygen, ~30% nitrous oxide, 2% halothane	27.32 ± 0.52[a]	38.93 ± 3.12	1.42 ± 0.12
2% halothane, air	31.25 ± 0.94[a]	—	—
4% halothane, air	33.72 ± 1.08[a]	—	—
Azaperon/etomidat and air	33.36 ± 0.76	43.36 ± 1.34	1.32 ± 0.06

[a] Data from van den Aardweg *et al.* (*9*).

TABLE II
Variation in ED_{50} (±SE) Values (Gy) for Moist Desquamation with Site in Two Strains of Pig Using Different Methods of Anesthesia for Irradiation with ^{90}Sr β Rays

		ED_{50} values (±SE)		
Strain	Anesthetic	Dorsal	Lateral	Ventral
English Large White	~70% oxygen, ~30% nitrous oxide, 2% halothane	28.18 ± 0.83[a]	26.76 ± 1.13[a]	26.46 ± 0.94[a]
	2% halothane, air	36.70 ± 1.34[a]	29.23 ± 1.37[a]	26.60 ± 1.51[a]
	4% halothane, air	37.55 ± 2.20[a]	32.66 ± 1.20[a]	30.47 ± 1.11[a]
	Azaperon/etomidat, air	36.28 ± 1.13	31.40 ± 1.21	30.44 ± 1.26
Göttinger Miniature	~70% oxygen, ~30% nitrous oxide, 2% halothane	44.06 ± 5.24	37.32 ± 3.44	36.03 ± 3.21
	Azaperon/etomidat, air	45.64 ± 2.13	43.57 ± 1.99	39.97 ± 2.15

[a] Data from van den Aardweg *et al.* (*9*).

dorsal and ventral skin fields ($0.1 < P < 0.2$). The use of azaperon/etomidat for anesthesia resulted in ED_{50} values of 45.64 ± 2.13 Gy and 39.97 ± 2.15 Gy for the dorsal and ventral skin fields, respectively. This difference did not quite reach accepted levels of significance ($0.05 < P < 0.1$).

DISCUSSION

In this study the radiation response of the epidermis was compared in two strains of pig, the English Large White and the Göttinger Miniature. The effects of two different conditions of anesthesia were also investigated. It is apparent from the results that the epidermis of the Göttinger Miniature pig is more resistant to radiation than that of the English Large White pig. The ratio of ED_{50} values was 1.42 ± 0.12 when the anesthetic used was ~70% oxygen, ~30% nitrous oxide, and 2% halothane, and 1.32 ± 0.06 for anesthesia based on azaperon/etomidat with the animals breathing air (Table I). Since these two ratios were not significantly different, a ratio of ~1.35 was taken to represent the difference in the radiosensitivity of the epidermis of these two strains of pig.

This difference in the radiosensitivity of the epidermis could be related to differences in the thickness of the epidermis in the two strains of pig. In the immature English Large White pig the depth of the basal layer of the epidermis on the flank was on average 60–90 μm.[1] In the Göttinger Miniature pig the thickness of the viable epidermal cell layers was 40 μm and the thickness of the stratum corneum ~70 μm, an overall epidermal thickness of ~110 μm (*11*). Variation in the depth-dose distribution for β rays can be considerable for target cells at different depths in the skin, but these variations are small for the superficial 200 μm (*12*). Thus it can be concluded for a given skin surface dose that the doses received by the target cell population were similar in these two strains of pig.

The difference in the radiosensitivity of the epidermis in the two strains of pig might be explained by the observed difference in the latent period for the development of moist desquamation. At the approximate ED_{50}, the isoeffect dose, the time of onset of moist desquamation was 46.5 ± 0.9 days in the Göttinger Miniature pig and 33.2 ± 1.1 days in the English Large White pig (*6*). The latent period for moist desquamation in the Yorkshire pig is even shorter, ~23 days (*13*). This difference in the time of onset of moist desquamation for these various strains of pig would appear to reflect differences in the natural turnover rates for the whole epidermis. In a previous publication (*4*) the time of onset of moist desquamation and the rate of cell loss from the basal layer of the epidermis were compared in the English Large White and Yorkshire pigs after single doses of X rays. The studies with Yorkshire pigs were carried out by Archambeau *et al.* (*13*). The time of appearance of moist desquamation in these two strains of pig was dependent on the normal rate of cell loss from the epidermis. This was faster in the Yorkshire pig (~4%/day) compared with 2.6%/day in the English Large White Pigs. The even later appearance of moist desquamation in the Göttinger Miniature pig suggests an even slower natural turnover rate of the epidermis and hence a much slower rate of cell loss after irradiation. These previous studies (*4*) also indicated that the turnover time of basal cells, as evidence of the initiation of attempted regeneration, was significantly shortened only when the density of cells in the basal layer was reduced by ~50%. This time of onset of attempted regeneration was at approximately half the latent period for the appearance of moist desquamation. This observation is also supported by the results of split-dose studies with varying intervals between fractions; an additional dose, to counteract the effects of recovery, was required when the interval between fractions was greater than approximately half that of the

[1] G. M. Morris, Epidermal cell kinetics in normal and X-irradiated pig skin. Ph.D. Thesis, University of London, 1987.

latency for the appearance of the measured effect (*5*). Once initiated, the capacity of the epidermis to regenerate and hence to avoid the development of moist desquamation is dependent on the number, turnover time, and the time available for proliferation of surviving clonogenic cells in the basal layer of the epidermis. Once accelerated proliferation is initiated, the turnover time of basal cells would appear to be very rapid, ~50 h (*4*). This may represent the maximum proliferation rate of clonogenic cells in the basal layer, and it is not unreasonable to assume that this would not be strain dependent. It is also unlikely that there is any marked difference in the inherent radiosensitivity of basal cells in animals of different strains. If these assumptions were valid, then a change in the time available for proliferation would have a profound effect on the apparent effectiveness of a given dose of radiation.

In the Yorkshire pig the period available for repopulation is between 12 and 23 days after irradiation; for the English Large White pig it is longer, 16–33 days. Pigs of the Yorkshire strain were apparently more radiosensitive than either of the two strains used in the present study. For example, a dose of 16.49 Gy in the Yorkshire pig evoked a similar cellular response to that produced by 20 Gy in the Large White pig (*4*). If the inferences from this comparison were extrapolated to the Göttinger Miniature pig, an even longer time period, 23–46 days, would be available for repopulation and hence a larger dose could effectively be tolerated before moist desquamation was seen to develop compared with pigs of the English Large White and Yorkshire strains. This would explain the apparent greater radioresistence of the epidermis in the Göttinger Miniature pig as was indicated by the higher ED_{50}, the isoeffect dose, for moist desquamation.

The studies carried out using azaperon/etomidat anesthesia with the animals breathing air resulted in higher ED_{50} values for moist desquamation in both strains of pig when compared with halothane/nitrous oxide anesthesia with the animals breathing ~70% oxygen. Previous information for azaperon/etomidat anesthesia has shown that this caused no significant change in the systolic and diastolic blood pressure of the pig when measured over a period of 75 min. There were also no detectable changes in cardiac function with azaperon/etomidat anesthesia (*10, 14*). However, both the arterial and venous hemoglobin concentrations were decreased significantly over a 75-min period. No change was detected in the oxyhemoglobin status over this period. These results suggest that anesthesia maintained with azaperon/etomidat is not likely to produce hypoxia, which may influence the radiosensitivity of tissues. However, anesthesia with azaperon/etomidat with the animals breathing air (~21% oxygen) did result in a decrease in the radiosensitivity of the epidermis in English Large White pigs compared with the use of a volatile gas anesthesia involving the use of ~70% oxygen. The ED_{50} for moist desquamation was 33.36 Gy, compared with 27.32 Gy. This decrease in the radiosensitivity of the epidermis is very similar to that reported previously for English Large White pigs irradiated using 4% halothane and with the animals breathing air; the ED_{50} value in this instance was 33.72 Gy (Table I). This slightly higher value might suggest a small reduction in the radiosensitivity induced by the azaperon/etomidat anesthesia, but the ED_{50} was not significantly different from that observed following the use of 2% halothane (ED_{50} 31.25 Gy). A similar change in the radiosensitivity of the epidermis was found for Göttinger Miniature pigs when the response of the animals anesthetized with the gas mixture was compared with those receiving azaperon/etomidat anesthesia and breathing air; the ED_{50} values were 38.93 Gy and 43.36 Gy, respectively. It can be concluded that the increased radioresistance of the epidermis in these two strains of pig when using the azaperon/etomidat anesthesia was caused mainly by the breathing of air during anesthesia.

Administration of azaperon/etomidat anesthesia while the animals were breathing air also caused a marked variation in the radiosensitivity of the epidermis over the flank in both strains of pig; dorsally positioned skin fields were radioresistant when compared with ventrally positioned fields. This result is also in agreement with previous studies which showed a variation in the radiosensitivity of the epidermis for animals breathing air mixed with 2% halothane (*9*). A reduction in the availability of oxygen to tissues induced by the infusion of a drug which enhances the affinity of oxygen for hemoglobin, produced the same effect (*15*).

Thus in summary it was concluded that the epidermis of the mature Göttinger Miniature pigs is apparently more radioresistant than that of the immature English Large White pig, a finding that was independent of the type of anesthesia used. However, this is more likely to reflect kinetic differences in the target cells of the basal layer, specifically the normal rate of cell loss, than any inherent difference in the radiosensitivity of those cells.

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