COMPARATIVE EFFECTS OF 50 KVP AND 250 KVP X RAYS ON THE DOG

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ABSTRACT

The subject of relative biological effectiveness of various ionizing radiations has been difficult and vexing to handle meaningfully when tissue distribution of dose is not uniform. It has been suggested by some that problems relating to linear energy transfer in tissues should appropriately be divided into two components - that relating to macroscopic energy distribution and that relating to energy distribution in terms of individual ionizing events. We have evaluated principally the former effect by looking at the biological potency of a lower energy X-ray source (50 kVp) in the dog.

Previously we have shown that no single dose parameter is adequate to express the biological potency of lower energy X rays, although it was suggested that, to the extent that it was possible to measure it, the dose to the critical organ, usually bone marrow, would be the most significant.

Further studies have been completed on dogs exposed to high doses of X radiation from a 50 kVp beryllium window generator. Doses of 4000 to 10,000 rad (air) at the potential midline of the subject have been shown to be as effective as doses of 200 to 300 rad of 250 kVp X rays. Hematological comparisons of the two radiations show close correlation with mortality and a relative potency factor of $\frac{1}{30}$ for the lower energy radiation. The lethal dose of 50 kVp X rays for the dog is 7500 r (air). Serious lesions of the skin were seen as a complicating factor at all doses in excess of 4000 r (air).

Dosimetry was done in tissue equivalent ("Mix D") wax phantoms using the rotational exposure method which is routine in this Laboratory. Phantom depth dose measurements with miniature ion chambers and chemical dosimetry in agar gels yielded essentially identical values of 3% for midline tissue dose.
SUMMARY

The Problem

Markedly heterogeneous distributions of ionizing radiation give rise to biological sequelae which generally are not predictable by physical dose parameters. It is the intent of this study to define physical dose distribution under both a uniform and a highly non-uniform condition and to observe and correlate the findings with physical dose measurement.

The Findings

Previously we have shown that no single dose parameter is adequate to express the biological potency of lower energy X-rays, although it was suggested that, to the extent that it is possible to measure it, the dose to the critical organ, usually bone marrow, would be the most significant.

Further studies on the effects of a highly heterogeneous distribution of radiation dose as the result of rotational exposure of dogs to 50 kVp X-rays has demonstrated that such exposure is nearly one-thirtieth as effective as an equivalent air dose of 250 kVp X-rays. It is also known that the bone marrow dose is still the best indicator of biological effectiveness of such exposures when the effect is related to bone marrow damage.

Serious lesions of the skin are seen following air doses of 4000 r.
INTRODUCTION

In previous publications in this series (1,2), it has been deduced that, for X rays in the sub-megavoltage range, the lethal effectiveness of the radiation is directly proportional to the dose received by the functionally limiting organ, usually the bone marrow. A number of units of dose measurement have been suggested and tested in order to provide a parameter which would predict with sufficient precision the biological effectiveness of unknown radiations (3,4,5), but none of these have proved to be completely successful.

The studies described here test the various dose measurements under conditions of extreme dose distribution inhomogeneity. Further, the 50 kVp radiation used has poor bone penetrating qualities compared with the 250 kVp radiation which is the comparison standard.

METHODS

Thirty-three mongrel male dogs weighing 7-15 kg on the day of radiation were employed in this study. The animals were quarantined for two weeks during which time they were dewormed and immunized against distemper and hepatitis. One week prior to the radiation exposure, the dogs were transferred to individual cages in a controlled environment animal room.

The dogs were exposed to seven doses (4000, 5000, 6000, 7000, 8000, 9000, and 10,000 r) at 50 kVp with four animals per dose for all except the 4000 and 10,000 r group which consisted only of one dog each, and to four doses (150, 200, 260, and 280 r) at 250 kVp with three animals per exposure dose.

One day post-radiation blood volume determinations were conducted on all dogs employing the T-1824 dilution method. At the same time approximately 20 µc of Fe59 was injected into the left jugular vein. On days 2, 3, 4, 8, 11, 16, 23, 30, 37, and 44, one ml samples of blood were withdrawn from the right jugular vein for isotope concentration measurements, erythrocyte, leukocyte, and thrombocyte counts, and hematocrit determinations. 0.250 ml of the blood was plated on a cupped planchet, and its radioactivity was measured with an end-window scintillation counter which had an over-all counting efficiency of approximately 4%.
IRRADIATION CONDITIONS AND DOSIMETRY

The animals were irradiated as described in a previous publication (1). Light pentobarbital anesthesia was induced by intravenous injection of the drug ten to fifteen minutes prior to commencement of irradiation. The animals were rotated about their long axis while held suspended in a canvas sling (3-4 rpm). This procedure permitted a uniform exposure of the surface of the animals and produced a distribution of dose in those animals exposed to the 250 kVp source which varied by no more than ± 5% throughout the phantom.

The air dose at the potential midline of the exposed animals was measured with bakelite or nylon wall Victoreen chambers for the 250 kVp and 50 kVp radiations respectively. Further, air dosimetry and depth dosimetry were done by means of firm gels incorporating chemical systems. These data are reported elsewhere (1,2,6,7).

Exposures to the 50 kVp source, which was a Picker "Zephyr" unit equipped with a Machlett OEG Beryllium window tube, were made with a 0.25 mm aluminum filter at 40 ma and a distance of 115 cm from the target to the potential midline of the exposed animals. The initial h.v.l. was 0.16 mm aluminum. Under these conditions the midline dose rate, in air, was 48 r/minute.

The 250 kVp exposures were made with a General Electric 250 kVp Maxitron operated at 25 ma. No filtration was added, but inherent tube filtration in this machine as used by us is relatively heavy. The initial h.v.l. is 1.8 mm copper and the ultimate h.v.l. is 2.7 mm copper. The dose rate to the potential midline of the dog at 115 cm from the target was 11.8 r/minute.

The ranking method of Wilcoxon (8) was utilized for statistical evaluation of the data.

RESULTS

A small decrease in Fe$^{59}$ incorporation was observed in dogs exposed to 4000 r, 50 kVp (Fig. 1). This depression approached, but was not quite as large as, that in dogs subjected to 150 r, 250 kVp. Since groups of animals which received 5000, 6000, and 7000 r did not vary significantly among themselves, their data were pooled. The mean isotope uptake of these groups was approximately 76% of the controls, which compares closely to the 80% obtained at 200 r, 250 kVp. Doses of 8000 and 9000 r, 50 kVp, which in all cases were lethal, depressed the iron uptake to 30% of normal and were similar to the incorporation obtained in dogs at 260 and 280 r, 250 kVp which was as
low as 36% of control values. The latter doses are in the region of
the LD50 for the higher energy. Here, as well as in the erythrocyte
counts and the hematocrit determinations, a greater decrease was
observed at 260 r than at 280 r.

No significant difference was noted in the erythrocyte count and
hematocrit determination at 4000 r, 50 kVp; 150 r, 250 kVp; and 200 r,
250 kVp (Fig. 2 and 3). The pooled value of 5000, 6000, and 7000 r,
50 kVp showed a significant decrease in the numbers of red cells from
a mean of $5.4 \times 10^6$ pre-radiation to a mean of $4.1 \times 10^6$ at day 23
post-radiation, while at the same time the hematocrit was reduced from
47% pre-radiation to 37% post-radiation (Fig. 2 and 3). Doses of 260
and 280 r at the higher energy showed comparative results, albeit the
depressions were slightly more protracted. The values at 8000 and
9000 r, 50 kVp were much further reduced and dropped to low values of
2.8 x 10^6 for erythrocytes and a hematocrit of 27% shortly before
death. Data obtained at 10,000 r cannot be evaluated because the
dog expired long before changes occurred in the peripheral blood
picture.

The leukocyte counts were depressed at all doses in both energies
in the early days post-radiation. 4000 r, 50 kVp was similar to 150 r,
250 kVp; 5000, 6000 and 7000 r, was similar to 200 r; 8000 and 9000
was similar to 260 r, and 10,000 r produced a decrease of a magnitude
comparable to that at 280 r. Figure 4 demonstrates the definite dose
dependence of the leukocyte counts at 50 kVp as well as at 250 kVp.
The pooled data of the 5000, 6000, and 7000 r, 50 kVp exposed dogs
presented an interesting picture. Commencing approximately 23 days
post-irradiation, the leukocyte count not only returned to pre-
irradiation values but, in fact, increased above them. This increase
ran parallel with the onset of skin lesions and infections caused by
absorption of the high radiation doses in the skin. Similar results
were not obtained at doses higher than 7000 r because of the death of
the dogs before the appearance of skin lesions.

Figure 5 clearly indicates an inverse relationship between the
number of platelets with increasing dose at 50 kVp. At the higher
energy such a relationship is not quite as apparent but it is present
to some extent. While platelet depressions at 5000, 6000, and 7000 r,
50 kVp (from $3 \times 10^6$ down to $0.7 \times 10^6$) were similar to those
observed at 150 and 200 r, 250 kVp (from $3 \times 10^6$ down to $0.5 \times 10^6$),
those at 8000 and 9000 r, 50 kVp ($3 \times 10^6$ down to $0.15 \times 10^6$) compared
closely to those at 260 and 280 r, 250 kVp ($3 \times 10^6$ down to $0.15 \times
10^6$).
The outstanding difference in the clinical pattern between dogs exposed to 50 kVp radiation and those receiving the higher energy radiation was the existence of moderate to extensive skin lesions in the former. However, the time of onset of these lesions occurred late enough that it had no significant modifying effect on mortality. Skin lesion appearance was at 15-30 days after exposure for any dose in excess of 5000 r. No lesions were seen in the animals receiving 4000 r.

Survival times for the animals succumbing to exposure of 8000 r, 50 kVp or more, were not significantly different from survival times reported by us (1) for higher energy radiations. At 8000 r the survival times ranged from 13-24 days. At 9000 r survival times were 12-20 days, and at 10,000 r the one dog survived ten days.

Since it is impractical to control the dose of the lower energy radiation closely enough to determine the LD50 by strict statistical procedures, the lethal dose is estimated from the knowledge that no dog succumbed from an exposure to 7000 r and no dog survived an exposure to 8000 r. We estimate, therefore, that the lethal dose is 7500 r.

A comparison has been made of the equi-effective doses of 50 kVp and 250 kVp radiation for a number of endpoints and the relative potency computed. These data are in Table I.

**DISCUSSION**

The quantitative endpoints measured in this study are in all cases indicators of damage to the blood-forming tissues, and lethality appears to be the result of bone marrow depression, as judged by survival time and clinical signs. Since we mentioned earlier in this report that it is most appropriate to examine the effectiveness of a given radiation in terms of the absorbed dose in the functionally limiting organ, we have made efforts to compare the absorbed dose in bone marrow necessary to produce similar effects with the two energies considered.

The absorbed dose in bone marrow will be determined by three factors, the attenuation by overlying soft tissue, attenuation by bone, and enhancement of dose in the marrow cavity close to the bone. At the energies used and for the size cavities being considered, two authors (9, 10) have deprecated the importance of dose build-up close to the bone/marrow interface. If dose is predicted on the basis of distribution in homogeneous phantoms, the principal error in estimating tissue dose will arise from bone attenuation. Wilson and
Carruthers (9) have measured the dose inside bony cavities in a human phantom for the energy range we are considering. They found that 0.5% of the air dose in roentgens was absorbed in marrow cavities of thoracic vertebrae and about 1.6% was absorbed in the marrow cavities of the sternum. They also deduced from an array of published data that the mean marrow dose required for an LD50 in the dog is 150 to 160 rad. We observed a ratio of approximately 30 between equi-effective air doses of 50 kVp and 250 kVp radiations for lethality. Predicting the 50 kVp LD50 from their data gives a value of 1000-3000 rad. The observed value is 7500 rad. The predicted bone marrow dose for lethality, based on our observed ratio of effectiveness, is 3% of the air dose or 225 rad, somewhat in excess of the 150 rad dose predicted by Wilson and Carruthers.

Since measurement of energy dissipation within the marrow cavity is not feasible and neither air, entrance, nor exit dose as suggested by various authors (3,4) are acceptable substitutes, it has become a recent tendency to adopt the midline "tissue" dose as suggested by Bond et al (5). This artificial measurement was obtained by serendipity, and as Alpen and Jones (1) so ably explain, it is a fortuitous resultant of increased bone attenuation and the rather superficial location of some bone marrow sites. For the present discussion, the midline tissue dose is reluctantly accepted as the closest available estimate of ionizing radiation actually occurring in the marrow cavity.

The midline dose obtained under the present experimental condition was 80% of air dose for 250 kVp X rays and 3% of air dose for 50 kVp X rays. According to Alpen and Jones (1) the LD50 for dogs is 275 r (air) at 250 kVp which gives us an attenuated dose in the center of 220 rad. This comes actually very close to our experimental findings of 212 rad and the predicted mean marrow dose of 225 rad.

It is plausible then to postulate that if bone marrow destruction is the main contributing factor to radiation death at the LD50 level, a ratio similar to that for the LD50 should be observed for tests of blood cell proliferation sites. The endpoints observed in the present study, erythrocyte and leukocyte estimations and hematocrit determinations, are now securely established sensitive indicators of hematopoietic radiation damage (2,11). Decreased Fe59 incorporation into newly formed erythrocytes of irradiated rats was first demonstrated by Hennessy and Huff (12) and the employment of this test in animals was subsequently defended experimentally and logically (13,14). The ratios obtained for hematological values (Table I) from the two X-ray energies deviate only slightly from that of the LD50, which seems to indicate a parallel course of destruction of blood
precursor cells and eventual death. In general, then, the hematological data of the present experiment appears to substantiate conclusions derived from studies at other radiation energies, that relative resistance to ionizing radiation of the premitotic blood cells in the bone marrow is the first line of defense for recovery.

An interesting feature of the present investigation is the apparent leukocytosis in dogs irradiated at 5000, 6000, and 7000 r, 50 kVp. This rise commences on day 16 post-radiation and returns to normal 20 days later. No increase is observed below 5000 r and obviously could not be detected at 8000 r since exposure at that level results in death no later than day 16 post-radiation. Concurrent with the increased number of peripheral leukocytes was the appearance of skin lesions. This latter fact permits some comparison with a study conducted by Brooks et al (15) on combined thermal injury and X irradiation (100 r, 1000 kVp) in dogs. In animals receiving a 20% contact burn alone, an early leukocytosis was observed reaching its peak on the 10th day. No such increase in peripheral white cells was noted when the dogs were subjected to 100 r X radiation in addition to the 20% burn. As a matter of fact, the count decreased until 17 days post-treatment when leukocytosis commenced in the surviving animals. The latter observation was similar to that which was seen in our dogs exposed to 5000, 6000, and 7000 r, 50 kVp, although we did not see skin lesions prior to day 14 and presumably the nature of the two skin injuries is quite different.
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<td>Lethality</td>
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Figure 1. Incorporation of radioactive iron ($^{59}\text{Fe}$) into peripheral erythrocytes. The tracer was injected on the first post-irradiation day.
Figure 2. Erythrocyte counts after exposure to graded doses of 50 kVp or 250 kVp X radiation.
Figure 3. Hematocrit determinations after exposure to graded doses of 50 kVp or 250 kVp X radiation.
Figure 4. Leucocyte counts after exposure to graded doses of 50 kVp or 250 kVp X radiation.
Figure 5. Platelet counts after exposure to graded doses of 50 kVp or 250 kVp X radiation.
REFERENCES


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Naval Radiological Defense Laboratory
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COMPARATIVE EFFECTS OF 50 KVP AND 250
KVP X RAYS ON THE DOG by S. J. Baum and E. L.
Alpen 24 January 1963 21 p. table illus. 15 ref.
UNCLASSIFIED

The subject of relative biological effectiveness of various ionizing radiations has been difficult and vexing to handle meaningfully when tissue distribution of dose is not uniform. It has been suggested by some that problems relating to linear energy transfer in tissues should be appropriately divided into two components - that relating to macroscopic energy distribution and that relating to energy distribution in terms of individual ionizing events. We have evaluated principally the former effect by looking at the biological potency of a lower energy X-ray source (50 kVP) in the dog.

Further studies have been completed on dogs exposed to high doses of X radiation from a 50 kVP beryllium window generator. Doses of 4000 to 10,000 rad (air) at the potential midline of the subject have been shown to be as effective as doses of 200 to 300 rad of 250 kVP X rays. Hematological comparisons of the two radiations show close correlation with mortality and a relative potency factor of \( \frac{1}{30} \) for the lower energy radiation. The lethal dose of 50 kVP X rays for the dog is 7500 r (air).

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