Induction of Osteosarcomas and Hematopoietic Neoplasms by $^{55}$Fe in Mice

Jean A. Laissue, Harold Burlington, Eugene P. Cronkite, and Ursula Reincke

Kantonsspital Luzern, Institute of Pathology, 6000 Lucerne, Switzerland [J. A. L.]; Department of Physiology, Mt. Sinai School of Medicine of the City University of New York, New York, New York 10029 [H. B.]; and Medical Research Center, Brookhaven National Laboratory, Upton, Long Island, New York 11973 [E. P. C., U. R.]

SUMMARY

Young, adult, female C57BL/6J mice received a single injection of $^{55}$FeCl$_3$ of high specific activity or equivalent amounts of cold iron. The animals were kept for duration of life to study late effects of $^{55}$Fe. The strain was selected because of known low tumor incidence and long life expectancy. The median survival times were 27, 117, and 439 days after treatment with 2.8, 1.4, and 0.7 mCi per mouse, respectively, and thus were inversely related to dose. Median survival in controls was more than 700 days. No tumors occurred in mice that survived less than 300 days. Six osteosarcomas developed in 14 mice that survived $^{55}$Fe treatment for more than 300 days. In addition, six other neoplasms were diagnosed: two leukemias, two thymic lymphomas, one hemangiendothelioma, and one reticulum cell neoplasm. Control mice had not reached their median survival time by Day 700 after treatment, and the only tumor noted was a reticulum cell neoplasm.

Radioiron was autoradiographically demonstrated in bone surfaces, bone marrow macrophages, and endosteal cells. Since osteosarcoma is believed to originate from the endosteum, it is conceivable that deposition within the target cell of $^{55}$Fe with its precisely located Auger electron radiation was instrumental in inducing neoplasms. Thus, identification of the cell at risk may be possible. Alternatively, but not exclusively, sufficient radiation for tumor induction may have been accumulated by the X-ray component of bone surface-seeking $^{55}$Fe.

INTRODUCTION

During a study of regulatory mechanisms in hematopoiesis with $^{55}$Fe cytocide as a major tool (14), we made a new and, we believe, interesting observation. We have noted a high incidence of osteosarcomas and neoplasias of the hematopoietic system in C57BL/6J mice treated with a single dose of $^{55}$Fe. Spontaneous osteosarcoma has not been recorded in this strain of mice.

MATERIALS AND METHODS

A total of 90 females (weighing 17 to 18 g) between 10 and 14 weeks of age received a single i.v. injection of 0.7, 1.4, or 2.8 $\mu$g iron, given as ferric chloride in 0.1 n HCl at the rate of approximately 1 $\mu$g iron per min. One-half of these mice were given injections of a solution containing $^{55}$Fe (specific activity, 1 mCi/$\mu$g iron, ICN Chemical Radioisotope Division, Irvine, Calif.), so that they received 0.7, 1.4, or 2.8 mCi of $^{55}$Fe. Experimental and control groups (15 mice in each dose group) thus differed only in regard to the injected radioactivity. They were housed 5 to a cage and fed ad libitum with Purina mouse chow and acidified drinking water (pH 2). Radioiron-treated mice were sacrificed only when moribund as judged by physical weakness, weight loss, or severe changes in blood cell counts. Control mice were killed either under the same circumstances or at intervals matching the reduced survival times of $^{55}$Fe-treated animals. At sacrifice blood counts and femoral bone marrow cell counts were obtained. All animals, with the exception of 2 $^{55}$Fe-treated and 4 control mice, were available for histopathological evaluation. At autopsy the following tissues were routinely removed and fixed in neutral buffered formalin (37 to 40% formaldehyde, diluted with 9 volumes of distilled water and brought to neutral pH with 20 g sodium acetate per liter): sternum, lumbar spine, femur, spleen, liver, thymus, lymph nodes, kidney, adrenal, intestine, pancreas, heart, lung, and skeletal muscle. Nondecalcified bones were embedded in methacrylate; other tissues were embedded in paraffin or methacrylate. Tissue sections were routinely stained with hematoxylin and eosin or exposed to potassium ferrocyanide for iron staining (7). Additional tissue sections were coated with autoradiographic emulsion (Eastman Kodak dipping emulsion NTB-2, diluted with an equal volume of distilled water), exposed for 4 days at 4°C, and then stained slightly with hematoxylin. Most of the tumors were diagnosed according to the classification of Murphy (12). Statistical evaluation of the data was based on a $\chi^2$ test modified by Smirnow and Dunin-Barkowski (17).

RESULTS

This study was undertaken because we conceived the $^{55}$Fe cytocide technique as a possible tool in testing the exhaustability of hematopoietic stem cells (15). Systematic data on hematological changes, life expectancy, and
causes of death were sought in order to provide background for future stem cell studies as well as information about possible late, internal radiation effects of $^{55}$Fe. This isotope decays to stable $^{55}$Mn with a 2.7-year half-life, emitting extremely short-ranged Auger electrons and a minor component of 5.9-keV X-rays.

The C57BL/6J mouse strain was selected because of its long life expectancy and low tumor incidence, particularly of leukemia. This was confirmed in our control group. Of the 45 cold iron-injected mice, 17 were sacrificed in good health and 4 were lost by accident before Day 300, leaving 24 animals at risk. The 1st natural death occurred 391 days after treatment. By Day 700, 5 control mice had died spontaneously (Table 1) and 14 remained at risk. Median survival thus will be well over 700 days after treatment. Except for 1 tumor, a reticulum cell neoplasm (type A), control mice displayed essentially normal organ histology.

In the $^{55}$Fe-treated groups, median survival times were 27, 117, and 439 days after 2.8, 1.4, and 0.7 mCi/mouse, respectively. All mice that died less than 300 days after $^{55}$Fe injection, including 8 that were accidentally killed during ether anesthesia, showed profoundly depleted hematopoiesis in bone marrow and spleen. Lymphoid tissues, especially thymus and spleen, were markedly atrophic. Death occurred after onset of rapidly progressing anemia. This followed extended periods where blood counts were moderately and stably reduced.

We have described early effects (14) and preliminary observations on the late effects (16) of $^{55}$Fe injection. Detailed accounts of hematological and pathological data are in preparation. At present, we wish to report on the incidence of neoplasms. Since the 1st mouse with tumor was autopsied 328 days after treatment, we list spontaneous deaths in all groups in Table 1 beginning 300 days after treatment.

Three mice survived the 1.4-mCi treatment for more than 300 days. All 3 developed osteosarcomas while showing nearly normal hematopoiesis in marrow and spleen. Eleven mice treated with 0.7 mCi survived considerably longer than 300 days. At autopsy, their marrow cellularity and spleen weight were moderately reduced. Nine of the 11 mice died of cancer. There were 4 leukemias, 3 osteosarcomas, 1 hemangioendothelioma, and 1 reticulum cell neoplasm (Table 1).

The occurrence of a total of 6 osteosarcomas was of particular interest since this tumor has not been seen in large colonies of untreated C57BL/6J mice over many years (H. J. Heiniger, The Jackson Laboratory, Bar Harbor, Maine, personal communication). The incidence of osteosarcoma in $^{55}$Fe-treated animals was significantly different from the zero incidence among controls ($p < 0.05$). All osteosarco-

Table 1
Incidence of neoplasms in experimental and control mice

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age of death* (days after $^{55}$Fe or cold Fe)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.7 mCi</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td></td>
</tr>
<tr>
<td>Lympoid leukemia</td>
<td>364</td>
</tr>
<tr>
<td>No neoplasm</td>
<td>370</td>
</tr>
<tr>
<td>No neoplasm</td>
<td>391</td>
</tr>
<tr>
<td>Granulocytic leukemia</td>
<td>407</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>414</td>
</tr>
<tr>
<td>Osteolytic sarcoma*</td>
<td>418</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>420</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>424</td>
</tr>
<tr>
<td>Pelvis cancer</td>
<td>436</td>
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<tr>
<td>No neoplasm</td>
<td>439</td>
</tr>
<tr>
<td>No neoplasm</td>
<td>444</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>449</td>
</tr>
<tr>
<td>Thymic lymphoma</td>
<td>450</td>
</tr>
<tr>
<td>Hemangioendothelioma</td>
<td>453</td>
</tr>
<tr>
<td>Thymic lymphoma</td>
<td>460</td>
</tr>
<tr>
<td>Reticulum cell neoplasm (type A)</td>
<td>462</td>
</tr>
<tr>
<td>No neoplasm</td>
<td>511</td>
</tr>
<tr>
<td>Pelvis cell neoplasm (type A)</td>
<td>679</td>
</tr>
<tr>
<td>At risk, 300 days after treatment</td>
<td>11</td>
</tr>
<tr>
<td>At risk, 400 days after treatment</td>
<td>9</td>
</tr>
<tr>
<td>At risk, 500 days after treatment</td>
<td>0</td>
</tr>
<tr>
<td>At risk, 600 days after treatment</td>
<td>0</td>
</tr>
<tr>
<td>At risk, 700 days after treatment</td>
<td>0</td>
</tr>
</tbody>
</table>

a The 1st tumor-bearing mouse died 328 days after injection. Therefore only deaths from Day 300 are listed here. Deaths from natural causes or sacrifice of moribund animals.

b Histologically analogous to osteosarcoma, but without neoplastic bone formation.

c Three mice sacrificed in good health.

d One mouse sacrificed in good health.
mas were localized in pelvis or femur. In some instances, metastases were noted in the liver and retroperitoneal space. Radiographs typically showed extensive destruction of the pelvic girdle and/or the femur as well as irregular dense zones. The latter corresponded to areas of neoplastic ossification in histological sections (Fig. 1). The sarcomas invaded adjacent soft tissues. Focal necrosis and hemorrhage were seen in most tumors. Fibrosarcomatous areas and multinucleated giant cells of the osteoclastic type were noted. Iron-positive pigment was usually absent within tumor tissue. One leukemic animal showed extensive accumulation of lymphoid blasts; the other showed proliferating and nonproliferating granulocytic cells mainly in bone marrow, spleen, liver, and lymph nodes. The thymic lymphomas and the reticulum cell neoplasms were also characterized by widespread involvement of tissues.

 Autoradiographic distribution of $^{55}$Fe in bone and bone marrow at the time of death revealed the following. (a) Silver grains were located primarily in areas with iron-positive pigment, mostly hemosiderin. (b) Accumulation of silver grains along periosteal and endosteal bone surfaces was seen (Figs. 2 and 3) and also occurred sometimes on the bone surface along Haversian canals. The number of silver grains in bone decreased with time after the injection of $^{55}$Fe. In long-term survivors, bands of silver grains were sometimes found buried in the depth of the cortical bone by apposition of new matrix (20). As the bands faded, more silver grains were found disseminated over cortical and trabecular bone. (c) Labeled endosteal and periosteal cells were occasionally noted in mice treated with 2.8 mCi (Fig. 4), whereas such cells were rare and only weakly labeled in the 1.4- and 0.7-mCi groups. Most of the label was seen over the cytoplasm. (d) Silver grains within the medullary cavities were mainly concentrated in “hot spots” (Figs. 2 to 4), overlying macrophages, reticulum cells, and sinus endothelia with larger amounts of hemosiderin. (e) Bone sections from animals given cold iron and processed for autoradiography together with those of radioactive mice displayed only a light diffuse background of silver grains without focal accumulation.

**DISCUSSION**

Osteosarcoma is not seen in untreated C57BL/6J mice, a strain with low tumor incidence and long life expectancy. Six osteosarcomas, as well as 6 other neoplasms, occurred among 14 mice of this strain that survived a single i.v. $^{55}$Fe injection for more than 300 days. The incidence is statistically significant. Since the presence of radioactivity was the only difference between experimental and control groups, neoplasms were evidently induced by radioactive decay. Neoplasms developed about 1 year after treatment and hence only after $^{55}$Fe doses permitting prolonged survival.

Extensive experiments on osteogenic sarcoma induction by bone-seeking radionuclides began during and after World War II (2). A large amount of data was collected. Work on radioactive metals, alkaline earths (strontium, radium), and actinide elements (thorium, plutonium) continues and is periodically reviewed (2, 8, 10, 18) and assessed (4, 9, 19).

Viruses may be involved in radiation-induced osteosarcoma (2, 6). Preneoplastic and degenerative changes in irradiated bone (5) are comprehensively summarized by Van Cleave (Ref. 21, Chap. 7).

Osteosarcoma due to $^{55}$Fe has not previously been reported. The findings were unexpected but not fortuitous. Discovery of any delayed $^{55}$Fe effects was one purpose of this study which was primarily designed to provide information on blood counts, life expectancy, and causes of death. The small number of mice allows no conclusions about the dose-incidence relationship. The radioactivity administered, 40 to 160 $\mu$Ci/g, was 1000-fold that which produced osteosarcoma by $^{85}$Sr (2). Other tumors diagnosed in the $^{55}$Fe-treated mice (Table 1) included a granulocytic leukemia. Bone-seeking radionuclides induce neoplasms elsewhere in the body (18), but the heterogeneity of tumor sites and types often precludes statistically satisfying evaluation.

Iron travels to bone as do the actinide elements, which also are bound to transferrin and ferritin (18). Incorporation of $^{55}$Fe into osteoid, trabecular bone, and marginal zone of the compact layer has been reported (3). Similarly, $^{55}$Fe incorporation into bone surfaces was demonstrated autoradiographically in the present study (Fig. 2). Months later, bands of isotope layer were buried within the cortical bone by apposition of new matrix. Thus iron behaves like plutonium (5, 8, 18) and belongs to the bone surface-seeking rather than volume-seeking elements. It is noteworthy that gross histological changes as seen in radium poisoning were not observed in bone free of osteosarcoma.

Radioiron was also found in macrophages, reticulum cells, sinus endothelia, and, of possible significance for osteosarcoma induction, in endosteal and periosteal cells. The radiation from $^{55}$Fe is precisely localized owing to its high proportion of low-energy Auger electrons which provide an autoradiographic range estimated to be 0.1 $\mu$m (13). We have calculated that a 300-cu $\mu$m cell receives about 0.25 rads per disintegration of incorporated $^{55}$Fe (14). Since the actual absorption volume hardly exceeds 1 cu $\mu$m for Auger electrons (1), damage is highly dependent on intracellular localization of isotope. Neighboring cells are not at risk from Auger electron irradiation but will be exposed to the 5.9-keV X-rays in a 90% range of about 1000 $\mu$m. The X-ray dose is very small compared to the Auger electron dose; we calculated an average of $3 \times 10^{-12}$ rads per disintegration to a 300-$\mu$m cell (14). However, the actual dose will vary with distance from the $^{55}$Fe source and may accumulate to significant values where $^{55}$Fe remains fixed for a substantial fraction of its 2.7-year half-life.

Osteogenic sarcoma is generally believed to originate within the interior of the affected bone, perhaps from the endosteum (4). The question then arises whether deposition within the target cell of $^{55}$Fe, with its short-range Auger electrons, was instrumental in inducing osteosarcoma. Alternatively, or perhaps as a combined effect, sufficient radiation for tumor induction may have been accumulated by the X-ray component of $^{55}$Fe deposited along bone surfaces. Bone surface-seeking radioisotopes, such as plutonium, thorium, yttrium, and americium, deliver higher...
doses to the bone surface than do bone-volume seekers such as alkaline earths, calcium, strontium, barium, and radium (8). It has been estimated that the probability of bone tumor induction by $^{89}$Sr was proportional to the square of the number of $\beta$ particles liberated in the skeleton of beagles, whereas the dose response for $\alpha$-emitters follows a more complicated pattern (9). The question of whether $^{55}$Fe incorporation into the target cell, general irradiation of the target cell, or a combination of both was responsible for bone tumors can only be answered by further experiments. If the 1st of the 2 possible mechanisms prevails, $^{55}$Fe could provide an interesting method for research on the cell at risk for carcinogenesis by bone-seek ing radioisotopes.

REFERENCES


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