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PULMONARY LESIONS INDUCED BY INHALED PLUTONIUM IN BEAGLES

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DISCLAIMER

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ABSTRACT

The histopathologic features of pulmonary fibrosis and bronchiolo-alveolar carcinoma in beagles exposed to aerosols of plutonium oxide were reviewed. A hypothesis of the pathogenesis of radiation pneumonitis induced by inhalation of plutonium oxide was presented; this hypothesis included phagocytosis of plutonium particles, fibrosis responding to the necrosis, and alveolar cell hyperplasia compensating for alveolar cells killed by alpha radiation. Histopathologic features of the epithelial changes suggest a progression from hyperplasia to metaplasia and, finally, to bronchiolo-alveolar carcinoma. The possibility of concurrent radiation-induced lymphopenia contributing to the development of bronchiolo-alveolar carcinoma through a loss of immunologic surveillance was discussed.

INTRODUCTION

The inhalation of plutonium is the most common route of contamination in man (Norwood, 1972). The inhalation of plutonium in rodents and dogs has been and is now being studied to predict the deposition, translocation, and biological effects of inhaled plutonium in man. This paper will review the published work and current data from studies at Battelle-Northwest Laboratories describing the pulmonary lesions induced by inhaled plutonium in beagle dogs and discuss the major stages of the pathogenesis of the lesions.

PNEUMONITIS

The first report of radiation pneumonitis induced by plutonium in beagles described lesions in 31 beagles that died 55 to 412 days after deposition of 0.1 to 0.9 μCi of $^{239}PuO_2$ per gram of lung (Park et al., 1962). The lungs had irregularly shaped, tan-gray, patchy, firm, fibrotic areas interspersed between small, pink, subpleural emphysematous areas. The cut surface was firm and dark red to brown; serosanguineous fluid was present. The bronchi contained a clear to serosanguineous fluid or white froth. The pale fibrotic areas were most prominent immediately beneath the surface of the lung and extended irregularly into the lung. The weights of exposed dog lungs were two to three times greater than the lung weights of the controls. The early histologic feature was partially organized deposits of fibrin in the alveoli. Fibrous thickening of alveolar septa was a prominent change in some animals. Large macrophages were present in the alveoli and were a principal site of deposition of plutonium particles. Small areas of alveoli were lined by simple columnar epithelium distinctly resembling the bronchioles, and squamous metaplasia of bronchiolar epithelium frequently occurred. Bronchial epithelium had abnormalities of epithelial cell size and shape or was denuded focally in advanced cases.

The sequence of developing histopathologic changes was further described in dogs sacrificed 1 to 30 days after exposure or those dying 55 days to 855 days with up to 27 μCi of $^{239}PuO_2$ in the lung (Clarke and Bair, 1964). These studies described the first changes as slight septal cell proliferation at 7 days postexposure with the additional appearance of a few scattered alveolar macrophages at 14 days. By 30 days there were multiple small areas of fibrosis in septal, peribronchiolar, and

perivascular areas; accumulations of macrophages in alveoli; and acute inflammation. Changes observed up to 855 days generally consisted of increasing amounts of septal and peribronchiolar fibrosis and metaplasia of alveolar and bronchiolar epithelial cells.

Radiation-induced lung changes were graded in a study involving exposure of beagles to varying lung burdens of either $^{239}PuO_2$ or $^{238}PuO_2$ (Yuile et al., 1970). The degree of radiation pneumonitis increased with increasing accumulated alpha doses above 1,500 to 2,000 rads and up to about 15,000 rads. The gross and microscopic descriptions were similar to those described by Park et al. (1972), Clarke and Bair (1964), and Park et al. (1964). The microscopic changes emphasized were (1) increased numbers of alveolar macrophages; (2) swelling, atypical proliferations, and metaplasia of alveolar and/or bronchiolar epithelial cells; (3) hyaline thickening of alveolar walls and small blood vessels; and (4) interstitial fibrosis.

The lung histopathology following exposure to $^{239}PuO_2$, $^{238}PuO_2$, and $^{239}Pu(NO_3)_4$ appeared similar for a comparable radiation exposure of these various chemical and physical forms of Pu (Howard, 1971). The life-shortening effects for $^{239}PuO_2$ were summarized according to the dose-effect relationship. Alveolar deposition of $^{239}PuO_2$ in amounts of 0.1 μCi/g of lung tissue or more was associated with respiratory failure in less than 1 month and up to 10 months, characterized as pulmonary edema, severe vascular damage, fibrinous accumulations in bronchioles and alveoli, and pulmonary fibrosis. Alveolar deposition in the range of 0.05 μCi/g of lung induced pulmonary fibrosis, bronchiolo-alveolar cell hyperplasia and metaplasia, alveolar histiocyte proliferation, pleural fibrosis and early

"alveolar tumor formation" in 1 to 5 years. Lung neoplasia was associated with death 3 to 10 years postexposure at doses of about 0.01 μCi/g of lung.

Additional changes accompanying the radiation pneumonitis are a dose-related lymphopenia (Park et al.; 1972) and sclerosed tracheobronchial lymph nodes (Clarke and Bair, 1964). The tracheobronchial lymph nodes accumulate much of the plutonium deposited in the lung and undergo a progression of changes resulting in the parenchyma finally being completely replaced by scar tissue at the higher dosage levels.

NEOPLASIA

Bronchiolo-alveolar tumors were first described in 4 dogs surviving more than 855 days after inhalation of $^{239}PuO_2$ with lung burdens at death of 0.6 to 1.4 μCi (Clarke et al., 1964). The lungs of these animals had marked amounts of radiation pneumonitis characterized as diffuse fibrosis, sclerosis, and in some instances hyalinized areas in subpleural, septal, and peripheral regions. The authors described a gradual transition from alveolar metaplasia to more anaplastic and finally neoplastic forms. The tumors were described as follows: "Infiltrating pleomorphic cells in these regions assumed epithelioid or squamous characteristics, formed frondlike papillary projections, or took the appearance of bronchiolar or acinar structures." The finding of these tumors prompted the authors to re-examine lungs of dogs from previous studies, and they identified similar epithelial changes that could be considered neoplastic in a dog that died 150 days after exposure with a lung burden at death of 19 μCi. It should be noted that the authors used the conservative diagnosis of "tumor" and "neoplasm" instead of "carcinoma" since, although local invasion was apparent, metastasis was not demonstrated.

Following the initial report of "bronchiolo-alveolar tumors," more pulmonary neoplasms were observed in ongoing studies of beagles exposed to $^{239}PuO_2$ (Clarke et al., 1966; Clarke, Park, and Bair, 1966). These were described as bronchiolo-alveolar carcinomas. The morphology of $^{239}PuO_2$-induced tumors in 22 dogs was described later (Howard, 1971) as mostly bronchiolo-alveolar carcinomas of peripheral origin, with two peripheral squamous cell carcinomas and three epidermoid carcinomas. Metastases were often demonstrated in the tracheobronchial and mediastinal lymph nodes and were found less often in the mesenteric lymph nodes, liver, kidney, spleen, adrenal glands, brain, and bone marrow. The high incidence of primary lung tumors was emphasized in a later paper (Park et al., 1972) when 20 of the 21 dogs that survived at least 4.5 years after $^{239}PuO_2$ exposure developed pulmonary neoplasia. The estimated accumulated average radiation dose to the lungs was 2,000 to 12,000 rads in these dogs. Bronchiolo-alveolar carcinoma, identical to that seen in $^{239}PuO_2$ dogs, has occurred in a $^{238}PuO_2$-exposed dog (Park et al., 1974). These reports are all from ongoing life-span studies. The morphology will be described in more depth in future publications.

PATHOGENESIS

Although the lesions of radiation injury due to plutonium have been described, the critical steps in the development of these lesions in dogs remain unknown. The most interesting feature in their development is the apparent progression of epithelial changes to neoplasia. At the dosage levels studied, the dogs either died from severe radiation pneumonitis or, at lower doses, less severe pneumonitis and pulmonary neoplasia.

The phagocytosis of plutonium particles occurs in alveolar macrophages (Adee et al., 1968; Sanders and Adee, 1970; Lutz et al., 1970; Nolibe, 1973), and alveolar type I cells of rats (Sanders and Adee, 1970). Although not as well documented, similar phagocytosis presumably occurs in alveolar macrophages and type I alveolar epithelial cells of dogs. Mitosis-linked cell death is a general phenomenon of radiation injury (Barenson et al., 1960) and is possibily the major response of cells to the 5.1 MEV alpha particles. This would result in cell death or small areas of necrosis, depending on the concentration of particles and local distribution.

If the particles are sequestered, either intra- or extra-cellularly, continued local injury would result, with eventual repair by fibrosis. Epithelial cell proliferation could result from continued radiation-induced cell death, or more likely, secondary to the focal fibrosis and chronic inflammation. Epithelial cell metaplasia in association with focal interstitial fibrosis attributed to lungworm infection has been reported in beagle dogs (Hirth and Hottendorf, 1973). Similar atypical or dysplastic epithelial cells have been observed secondary to fibrotic lesions resulting from a variety of known and unknown causes in man. In some cases, transition to carcinoma has been reported (Fraire and Greenberg, 1973). It is interesting and perhaps of significance that one-third of the lung carcinomas in man reported to be associated with fibrosis have been classified as bronchiolo-alveolar cell types, whereas this type accounts for only 3 - 6% of total lung tumors in man (Fraire and Greenberg, 1973). This is the predominant tumor observed in the Pu-exposed dogs. The stimulus for epithelial cell proliferation and transformation in association with fibrosis is not known and, at present, the existence of such a

stimulus is only speculative. The possibility that fibrosis may render the proliferating epithelial cells more susceptible to chemical or physical carcinogenesis cannot be excluded.

In summary, we have attempted in this workshop session to review the current status of Pu-induced pulmonary neoplasia and offer some thoughts as to its pathogenesis. There are many gaps in the knowledge of the steps which occur in the carcinogenic process. Studies on the immunologic competence of the dogs in relation to the lymphopenia observed after Pu exposure and prior to the appearance of neoplasia have been initiated. Electron microscopic studies will aid in the identification of the cells of origin of the neoplasms and more detailed studies concerning the spatial distribution of the Pu particles will assist in evaluating dose-effect relationships. A study comparing the low-level effects of soluble vs. insoluble forms of plutonium has recently been initiated.

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