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Diffuse and continuous cell proliferation enhances radiation-induced tumorigenesis in hamster lung

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Summary

Syrian Golden hamsters received 8 weekly intratracheal instillations of 0.2 μCi of the alpha-emitting isotope Po^{210} while being exposed to an atmosphere of 65% oxygen in the inspired air. Three months later, 42% of the animals had poorly differentiated lung carcinomas. On the other hand, no lung tumors were found in hamsters that received intratracheal instillations of Po^{210} and were kept in air. It is concluded that diffuse cell hyperplasia in the lung, caused by an inhalant, may constitute an additional risk factor in the pathogenesis of alpha-radiation induced lung cancer.

Keywords: lung tumors; hamsters; Po^{210}

Introduction

Radon and its products are known to cause lung cancer in man. Numerous epidemiological studies of underground miners have shown a very strong association between exposure to the alpha-particles emitting isotopes and development of malignant lung tumors. Cigarette smoking appears to increase the risk. It is also suspected, although not established unequivocally, that the presence of radon and its products in homes constitutes a human health hazard [1].

In animal models that imitate the human disease, the development of lung tumors is often modulated by inhalants, solutions instilled directly into the respiratory tract and by dietary factors. Enhancement of tumor development appears to be closely linked to increased and sustained cell hyperplasia in the respiratory tract and tumors are often localized in areas of most intensive cell proliferation (reviewed in Ref. 2). For example, in hamster lung exposure to hyperoxia enhances the development of tumors of neuroendocrine cell origin [3,4]. On the other hand, inhalants also may suppress respiratory tract tumor formation, even when capable to elicit a hyperplastic response. Examples are ozone and oxygen in mouse and rat lung and NO_2 in hamster lung [5 – 8]. In light of these observations it was of interest to examine the effects of cell proliferation caused by chronic hyperoxia on the development of lung tumors produced by Po^{210}, an accepted model for radon carcinogenesis [9]. The question asked was to what extent radiation carcinogenesis might be susceptible to modulation by an inhalant. The

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results of our experiment show that hyperoxia is capable to accelerate lung tumor development in hamsters.

Materials and Methods

Animals

Outbred male LVG Syrian Golden hamsters, purchased from Charles River and 8 to 10 weeks old at the beginning of the experiment were used. They were housed in our accredited facilities in polycarbonate cages on hardwood bedding and had free access to conventional lab chow and water throughout the experiment.

Radiochemical

Po^{210}, 1 mCi in 5 ml of 3 N nitric acid was purchased from Amersham-Searle. The stock solution was diluted with 0.9% NaCl to a final concentration of 1 μCi/ml and the pH adjusted to 5.0 with NaOH.

Experimental procedures

All experimental procedures were approved by the Committee on Animal Welfare, University of California, Davis. Intratracheal instillations were performed under methoxyflurane anesthesia. One group of animals received 8 weekly instillations of 0.2 μCi of Po^{210} intratracheally while being kept continuously in a chamber ventilated with an atmosphere of 65% oxygen; details of the exposure system have been described [5]. After the last instillation, oxygen exposure was continued until the animals were killed. A second group received 8 instillations of Po^{210} and was kept in air throughout. Control groups were animals kept in 65% oxygen or in air and given 8 intratracheal instillations of 0.9% NaCl. Animals were killed when visibly sick (body wt. falling below 80 g) or at the end of the experiment, 3 months after the first instillation.

A complete autopsy was done and the lungs were fixed with neutral buffered formalin by vascular perfusion through the portal vein under controlled pressure. Lung tissue was embedded in paraffin, cut and stained with H&E. Immunoperoxidase stains for calcitonin and mammalian bombesin were done using the Vectastain ABC-kit using DAB as substrate and hematoxylin as counterstain, as described before [3,4]. The number of animals bearing lung tumors was recorded and statistical comparisons between animals kept in hyperoxia and animals kept in air was done by using the χ^2-test.

Results

All animals treated with a cumulative dose of 1.6 μCi of Po^{210} and kept in air gained weight and survived until the scheduled sacrifice 3 months after the beginning of the experiment. The animals treated with Po^{210} and exposed to the hyperoxic environment kept their initial weight during the first 6 weeks. They then began to loose weight; 7 out of 24 animals were found dead or had to sacrificed because of poor clinical conditions after about 2.5 months. Hamsters treated with instillations of 0.9% NaCl and kept in 65% oxygen also lost weight towards the end of the experiment and 3 animals died 2 to 3 weeks before the end of the experiment. All animals instilled with 0.9% NaCl and kept in air survived. The incidence of all histologically verified lung tumors is listed in Table I.

The lung tumors were highly cellular with little stroma. Formation of glandular structures and/or evidence of secretion suggestive of differentiation into adenomas/adenocarcinomas was missing. Likewise, there was no evidence of keratin formation or squamous differentiation suggestive of differentiation into squamous cell carcinomas. The tumor cells were spindle-shaped with a large nucleus/cytoplasmic ratio, a morphology frequently found in small cell lung cancers. The tumors demonstrated an aggressive growth pattern invading local blood vessels and bronchiolar walls (Fig. 1). However, immunostains for the most common neuroendocrine markers of this tumor, calcitonin and mammalian bombesin, were negative. Based on their aggressive growth pattern in conjunction with a lack of

Table I. Incidence of lung tumors in hamsters treated with intratracheal instillations of Polonium-210.

Treatment	No. of tumor bearing animals/ % total no. of animals per group	%
Hyperoxia and Po^{210}[a]	10/24[b]	42
Air and Po^{210}[a]	0/18	0
Hyperoxia and 0.9% NaCl[c]	0/8	0
Air and 0.9% NaCl[c]	0/10	0

[a]Animals were given 8 weekly intratracheal instillations of 0.2 μCi of Polonium-210 and kept in either 65% oxygen or in air.

[b]Lung tumor incidence statistically significant ($P < 0.05$) from animals kept in air.

[c]Animals received intratracheal instillations of 0.9% NaCl.

neuroendocrine, squamous or adenomatous markers, the tumors were diagnosed as poorly differentiated lung carcinomas.

Discussion

Our findings show that in hamsters given a cumulative dose of 1.6 μCi of Po^{210} intratracheally and kept in a hyperoxic environment (65% oxygen), multiple peripheral lung tumors develop within 2 to 3 months in 42% of the animals. No lung tumors were found within the same time period in hamsters that were exposed to an equal dose of Po^{210} and were kept in air. Chronic hyperoxia is known to produce diffuse cell hyperplasia in both the bronchial tree and the lung parenchyma [4,5]. Some years ago, Little et al. [10] reported that the intratracheal instillation of the apparently inert solution of 0.9% of NaCl into the respiratory tract enhanced Po^{210} carcinogenesis, presumably because it caused cell proliferation. A more recent study reemphasized the role of cell proliferation caused by wounding or intratracheal saline instillations in enhancement of respiratory tract carcinogenesis in hamsters [11,12]. Our observations confirm that tumor development also can be enhanced by an inhalant such as oxygen.

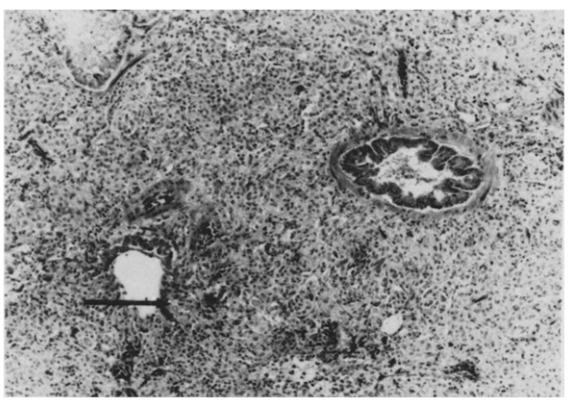

Fig. 1. Poorly differentiated carcinoma induced by Po^{210} and hyperoxia. The compact tumor demonstrates disorganized morphology including areas with endocrinoid growth pattern and palisading spindle shaped cells. Local invasiveness (destruction of bronchus by tumor cells, arrow) indicates highly malignant potential.

In previous experiments we have documented that hyperoxia produces within a few weeks not only a general hyperplastic response in the respiratory tract [5] but also causes a substantial proliferation of a particular lung cell population, the neuroendocrine cells [4]. In conjunction with a chemical carcinogen, this event results in the development of multiple lung tumors of neuroendocrine differentiation [3,4]. In the present investigation, many of the tumors found in the lung periphery resembled, under the light microscope, the tumors found in previous studies. However, in the absence of positive immunstains we were unable to make a definite diagnosis and the tumors were classified as poorly differentiated lung carcinomas.

Our present observation may be of some importance for risk assessment. Pulmonary diffuse cell hyperplasia may constitute a contributing risk factor in the development of radiation-induced lung cancer. In the present experiment we have shown that hyperoxia is a risk factor that shortens the time to tumor. It is not likely that hyperoxia plays a role in the development of human lung cancer. However, it is known that proliferation of the pulmonary epithelial cell population may occur under circumstances that directly apply to the human situation. Many toxic air pollutants elicit a proliferative response in the respiratory tract [13]. Pulmonary neuroendocrine cell hyperplasia has been found in the lungs of people suffering from chronic lung disease [14] and in experimental animals kept in hypoxia [15], ozone [16] NO_2 [17], or exposed to cigarette smoke [18]. The effect of a toxic, although not carcinogenic inhalant, may thus modify lung tumorigenesis by the alpha-particles emitting radioisotope Po^{210} and change the risk to develop lung cancer from internal radiation. Risk assessment in humans relies on epidemiological data. The contribution of pulmonary cell proliferation caused by inhalants as an additional factor is probably already included in such estimates. On the other hand, risk assessments based on animal studies that rely on radiation dosimetry alone may have the potential of underestimating risk increased by such compounding factors as cell proliferation.

In conclusion, our studies have shown that hyperoxia can act as a promoting stimulus for radiation-induced lung carcinogenesis. Whether or not this is caused by neuroendocrine cell proliferation or other factors will have to be determined in future studies.

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References

1 BEIR IV Report (1988) The Effects of Radon and Other Internally Deposited Alpha-Emitters, Appendix III: The Effects of Radon Progeny on Experimental Animals. National Research Council, National Academy Press, Washington DC.

2 Witschi, H. (1990) Modulation of Lung Tumor Development in Rodents. Prog. Exp. Tumor Res., 33, 132 – 153.

3 Schuller, H.M., Becker, K.L. and Witschi, H.P. (1988) An animal model for neuroendocrine lung cancer. Carcinogenesis, 9, 293 – 296.

4 Schuller, H.M., Witschi, H.P., Nylen, E., Joshi, P.A., Correa, E. and Becker, K.L. (1990) Pathobiology of lung tumors induced in hamsters by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and the modulating effect of hyperoxia. Cancer Res., 50, 1960 – 1965.

5 Lindenschmidt, R.C., Tryka, A.F. and Witschi, H.P. (1986) Inhibition of mouse lung tumor development by hyperoxia. Cancer Res., 46, 1991 – 2000.

6 Lindenschmidt, R.C., Margaretten, N., Griesemer, R.A. and Witschi, H.P. (1986) Modification of lung tumor growth by hyperoxia. Carcinogenesis, 7, 1581 – 1586.

7 Last, J.A., Warren, D.L., Goad, E.P. and Witschi, H.P. (1987). Modification by ozone of lung tumor development in mice. J. Natl. Cancer Inst., 78, 149 – 154.

8 Witschi, H.P., Breider, A.M. and Schuller, H.M. (1990) Modulation of *N*-nitrosodiethylamine induced hamster lung tumors by nitrogen dioxide. Inhal. Toxicol. (in press).

9 Little, J.B. and Kennedy, A.R. (1979) Evaluation of alpha radiation-induced respiratory carcinogenesis in Syrian hamsters: total dose and dose-rate. Prog. Exp. Tumor Res., 24, 356 – 369.

10 Little, J.B., McGandy, R.B. and Kennedy, A.R. (1978) Interactions between Polonium-210 alpha-radiation, Benzo(a)pyrene, and 0.9% NaCl Solution Instillations in the Induction of Experimental Lung Cancer. Cancer. Res., 38, 1929 – 1935.

11 Keenan, K.P., Saffiotti, U., Stinson, S.F., Riggs, C.W. and McDowell E.M. (1989) Morphological and cytokinetic responses of hamster airways to intralaryngeal or intratracheal cannulation with instillation of saline or ferric oxide particles in saline. Cancer. Res., 49, 1521 – 1527.

12 Keenan, K.P., Saffiotti, U., Stinson, S.F., Riggs, C.W. and McDowell, E.M. (1989) Multifactorial hamster respiratory tract carcinogenesis with interdependent effects of cannula-induced wounding, saline, ferric oxide, benzo(a)pyrene and N-methyl-N-nitrosourea. Cancer Res., 49, 1528 – 1540.

13 Bils, R.F. and Christie, B.R., (1980) The experimental pathology of oxidant and air pollution inhalation. Int. Rev. Exp. Pathol., 21, 195 – 293.

14 Becker K.L. (1984) The Endocrine Lung. In: The Endocrine Lung in Health and Disease, pp. 2 – 46. Editors: K.L. Becker and A.F. Gazdar. W.B. Saunders, Philadelphia.

15 Pack, R.J., Barker, S. and Howe, A. (1986) The effect of

hypoxia on the number of amine-containing cells in the lung of the adult rat. Eur. J. Respir. Dis., 68, 121 – 130.
16 Castleman, W.L., Dungworth, D.L., Schwartz, L.W. and Tyler, W.S. (1986) Acute respiratory bronchiolitis. An ultrastructural and autoradiographic study of epithelial cell injury and renewal in Rhesus monkeys exposed to ozone. Am. J. Pathol., 98, 811 – 840.
17 Kleinerman, J., Marchevsky, A.M. and Thornton, J. (1981) Quantitative studies of APUD cells in airways of rats. The effects of diethylnitrosamine and NO_2. Am. Rev. Respir. Dis., 124, 458 – 462.
18 Tabassian, A.R., Nylen, E.S., Linnoila, I.R., Snider, R.H., Cassidy, M.M. and Becker, K.L. (1989) Stimulation of hamster pulmonary neuroendocrine cells and associated peptides by repeated exposure to cigarette smoke. Am. Rev. Respir. Dis., 140, 436 – 440.