Dose repartition in alveoli, alveolar ducts and bronchi of rats exposed to radon and its progeny, preliminary results

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DOSE REPARTITION IN ALVEOLI, ALVEOLAR DUCTS AND BRONCHI OF RATS EXPOSED TO RADON AND ITS PROGENY, PRELIMINARY RESULTS.

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Abstract

Rats were exposed to 2 ppm ozone for 5 hours to increase micronuclei formation in the different lung compartments one week after local irradiation. Animals were exposed to either radon and its progeny (300 and 1000 WLM) or local lung external irradiation using gamma rays from cobalt-60 (7.5 Gy). Animals were administered 5-bromo 2'deoxyuridine 18 and 12 hours before to label post replicative cells. They were killed 36, 48 and 60 hours after the beginning of the ozone exposures. Alveolar macrophages (AM) were recovered by lavage of the left lobe and the right lobes were fixed for histological studies using confocal microscopy.

Micronuclei measurements in AM did provide only mean dose estimate to the deep lung. Histological observations showed a nearly homogeneous irradiation by gamma rays whereas, heterogeneous irradiation seemed to occur after radon exposure with a gradual dose increase from alveoli to bronchi.

Confocal microscopy/ Micronuclei/ Radon/ Ozone/ Pulmonary epithelial cells/ Alveolar macrophages/ Rats/ Gamma rays/

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1. Introduction

Recently, micronuclei scoring has been performed to estimate doses delivered to the lungs after heterogenous irradiation induced after inhalation of radon and its progeny. These studies were limited to the deep lung after either extraction of alveolar macrophages (AM) by lavage (Taya et al., 1994, Johnson and Newton, 1994) or enzymatic dissociation of lung cells to initiate fibroblast cultures (Khan et al., 1994). Dose estimates were performed after comparison with micronuclei formation induced *in vitro* by irradiation with alpha particles.

We have previously shown that, after irradiation, micronuclei formation can be greatly increased by an acute ozone exposure which transiently stimulates lung cell proliferation, mainly for epithelial cells and AM (Bisson et al 1994), we have also developed an experimental procedure to score micronuclei in post-replicative cells on lung thick sections by scanning confocal microscopy (Bisson et al., 1995).

The aim of this study was to provide qualitative and quantitative results to compare micronuclei induction in AM after radon and gamma ray exposure and to estimate dose distribution within the different lung compartments.

2. Material and methods

Male Sprague-Dawley rats (OFA, Iffa Credo, France), were used at 3 months of age. Five groups of animals were studied : rats exposed 1) to ozone alone, 2) to gamma rays from cobalt-60 and ozone, 3) to radon and ozone, 4) to radon alone and 5) unexposed controls. The irradiations were performed one week before the ozone exposure.

Ozone exposure was whole-body performed in a Hazleton inhalation chamber. Ozone was produced by passing pure oxygen through a silent electric arc-type ozoniser (WALLACE and TIERNAN BA 023012). The gas mixture was diluted with air to obtain a final concentration of 2 ppm which was monitered continously in the chamber with an analyser (O_3 43M, Environnement S.A.). The duration of exposure was 5 hours (Trédaniel et al., 1994).

Whole-body exposure to radon and its progeny was performed during 24 hours in the Razes facility to obtain either 300 or 1000 WLM (equilibrium factor : 0.80, "unattached" fraction : 0.08) (Monchaux et al., 1994). 7.5 Gy local lung gamma irradiation was performed using cobalt-60 at a dose rate of 1 Gy/min.

5-bromo 2'-deoxyuridine (BrdU) (25mg/kg) and deoxycytidine (10mg/kg) were administered 18 and 12 hours before killing to label cells which synthesize DNA. After pentobarbital anaesthesia, the left lung was lavaged, cells were counted and cytospin were observed after Giemsa staining, the right lobes were fixed in 4 % buffered paraformaldehyde for 3 days. Thick cryostat sections (80 µm) of fixed tissue were stained by a primary antibody mouse anti BrdU and

a secondary antibody: goat anti mouse FITC labelled. Cells were counterstaining by propidium iodine. Labelled cells were observed by scanning confocal microscopy as previously described (Bisson et al., 1995).

3. Results

In each group of irradiated rats, a nearly two-fold increase in the number of extracted AM was observed from 36 to 60 hours after the beginning of ozone exposure. This increase was similar to that observed in unirradiated controls. Figure 1 shows the percentage of AM with micronuclei (MiAM) in irradiated rats either exposed or unexposed to ozone. In control rats unexposed to ozone, MiAM was 0.2 % ± 0.1 which was not significantly modified by the ozone exposure. In irradiated rats unexposed to ozone, similar MiAM were observed for 7.5 Gy and 1000 WLM but, the ozone exposure induced a larger increase of MiAM for 7.5 Gy than for 1000 WLM. For all post ozone exposure times studied, MiAM induced after 300 WLM was always less than that induced by 1000 WLM.

Observations by scanning confocal microscopy of micronuclei in alveolar tissue seemed to be in agreement with results obtained in extracted AM. By contrast, in the bronchial epithelium, the fraction of cells with micronuclei appeared similar for 7.5 Gy and 300 WLM and most of the cells had only one micronucleus. A larger fraction of micronucleated bronchial cells seemed to occur for 1000 WLM than for 7.5 Gy and 300 WLM. Because most micronucleated cells after 1000 WLM contained more than 1 micronucleus, we have measured in one animal the number of micronuclei per micronucleated cells in different lung compartments. Results are shown in figure 2. About 50 %

of micronucleated alveolar cells contained only one micronucleus whereas, in alveolar ducts and in bronchi, this population corresponded to 43 % and 33 % of the total micronucleated cells respectively. The percentage of cells containing 4 micronuclei was higher in bronchi than in alveolar ducts or in alveoli, 25 %, 10 % and 5 % respectively .

4. Discussion

This study on AM confirms that ozone exposure increases micronuclei formation in irradiated rats and could be a useful tool for biological dosimetry (Bisson et al., 1994). The observed results did not provide an accurate value of dose equivalent for radon exposure but were consistant with that previously reported (1 WLM = 0.8 mGy alpha) (Khan et al., 1994) taking into account for micronuclei induction a relative biological effectiveness of 10 for alpha versus gamma irradiation (Brooks et al., 1994). Further studies are in progress to determine dose effect relationship for both homogeneous gamma irradiation and heterogeneous radon exposures, and the evolution of cellular alterations (spontaneous and ozone induced AM proliferation, micronucleated AM and multinucleated AM) as a function of post-irradiation time.

Although only preliminary results were obtained, the histological study provides new data for dose distribution estimate within lung tissues. Under the experimental exposure used, i.e. high equilibrium factor and low "unattached" fraction, a gradual increase of doses appeared to be delivered from the most distal lung to the bronchi. Thus, the maximal dose might not be delivered to the main target area for lung tumour induction which is located in the bronchiolo-alveolar junction (Poncy et al., 1992) or in alveoli (Cross et al., 1984).

These results are conflicting with dose repartition reported using mathematical model (Hofmann et al., 1993). Further studies are in progress to provide more complete quantitative results and to determine the effect of exposure conditions to radon and its progeny on dose distribution in the different lung compartments.

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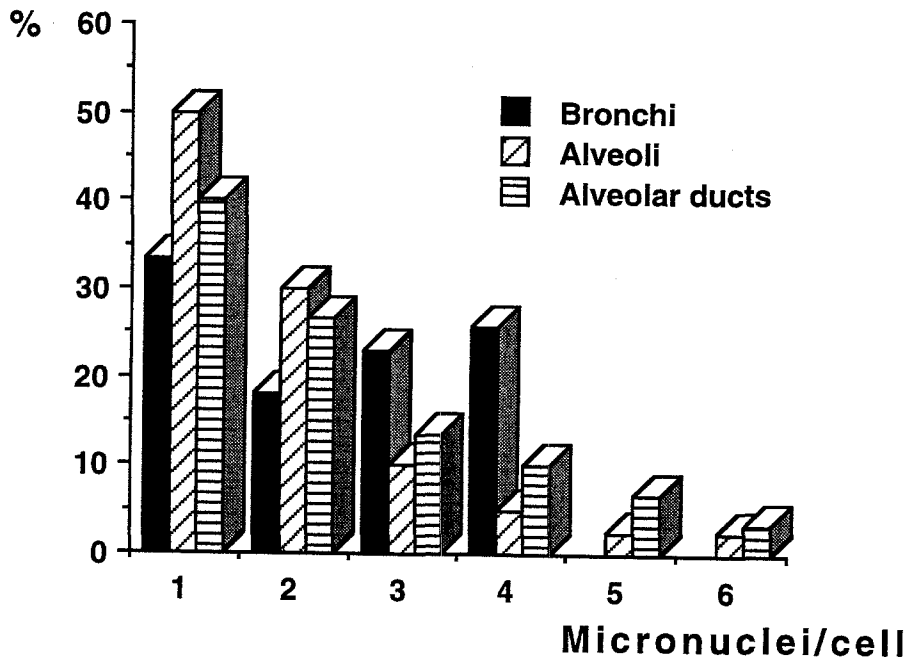
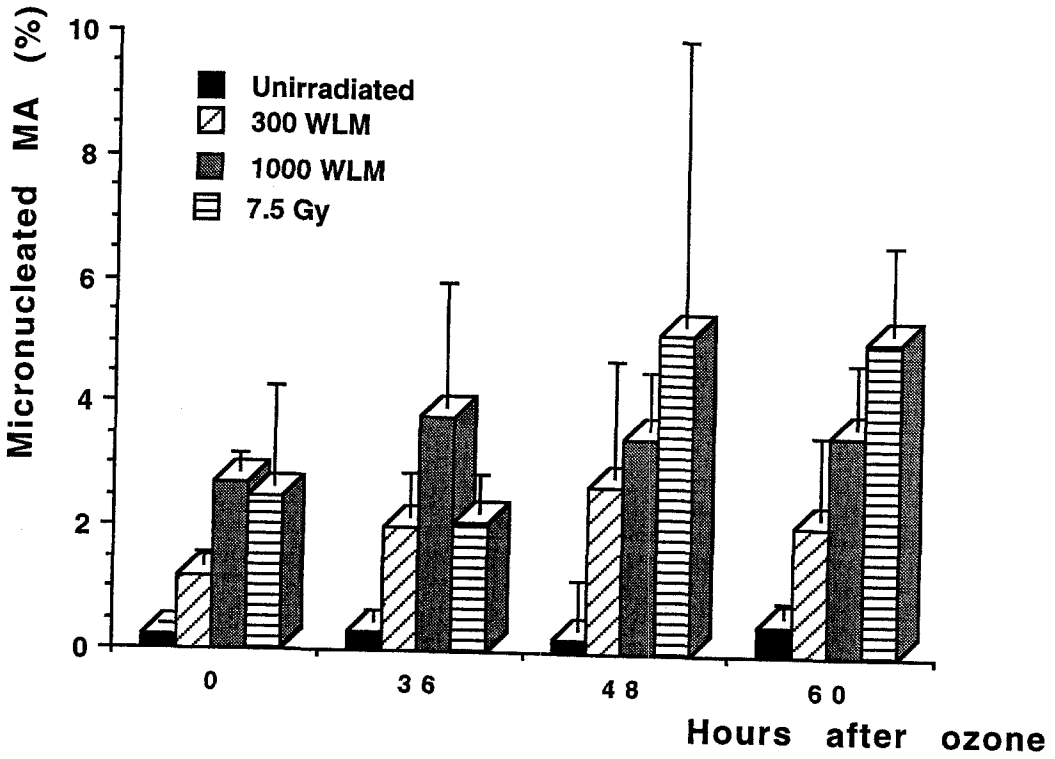
CAPTIONS

Figure 1: Evolution of micronucleated AM as the function of time following acute ozone exposure (mean values ± sd for at least 4 animals per group).

Figure 2: Distribution of the number of micronuclei per cell in the different lung compartment (animal exposed to ozone one week after 1000 WLM and killed 60 hours later).

Figure 3: Micronuclei within post-replicative cells of the bronchiolar epithelium. Observation performed by confocal microscopy after an exposure to 300 WLM and 48 hours after the ozone exposure (step between 2 successive view 2 μm).


%
60
50
40
30
20
10
0
Bronchi
Alveoli
Alveolar ducts
1
2
3
4
5
6
Micronuclei/cell


Micronucleated MA (%)
10
8
6
4
2
0
Unirradiated
300 WLM
1000 WLM
7.5 Gy
0
3 6
4 8
6 0
Hours after ozone