CORRELATION OF BRONCHOALVEOLAR LAVAGE AND COMPUTED TOMOGRAPHY IN AN EXPERIMENTAL MODEL OF SILICOSIS*

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Modern tunnel construction involves health hazards such as quartz-dust and compressed air. Compressed air is used behind air-tight bulk-heads to control water seepage. Applying shotcrete lining methods, considerable amounts of quartz-containing respirable dust might be generated.[1] Thus shotcrete tunneling in a compressed air environment eventually exposes the underground workers to both silicogenic and hyperbaric conditions. In addition, exposure to compressed air always means exposure to hyperoxic conditions. Regarding the current concepts of the pathogenesis of silicosis,[2] the interactions between silica particles and alveolar macrophages might be altered by hyperoxia, finally leading to modifications in the development of silicotic lung fibrosis. Moreover, the deposition of respirable dust particles might be changed due to hyperbaric effects on lung function.[3]

The effects of short-term exposure on various parameters of lung function had been described in miners working with shotcrete in a compressed air environment.[4] Thus, the aim of our study was to investigate the individual and combined effects of long-term exposure to silica and compressed air in a non-human primate model. In a longitudinal study (26 months of exposure) we examined various parameters, using broncho-alveolar lavage (BAL) cytological and functional assessment of free lung cells, biochemical analyses of BAL fluid, lung function tests, radiological (X-ray, CT-scan) and pathohistological examinations as chief methods. This paper will focus on the correlation of cellular constituents of BAL and quantitative analysis of CT-scans of the lung.

METHODS

Animals

Thirteen months prior to the start of exposure, 28 cynomolgus monkeys (Macaca fascicularis; 4 male, 24 female) with a body weight of approximately 3–6 kg were separated into four groups. Previously, the animals had been kept in quarantine, dewormed, and tuberculin-tested. In exposure-free intervals, the animals were housed in spacious steel cages under natural daylight. A standard primate chop diet, additional fruit supplement, and tap water were supplied ad libitum.

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Experimental Design

Following an acclimatization period of 6 months, control BAL was performed three times in each animal. After the start of exposure, BAL was carried out at intervals of 2 months. Open lung biopsies were taken 12 and 18 months after the start of exposure. After 26 months, the exposure was ceased. In the following two months, various lung function tests and radiological examinations (X-ray and CT-scan) were performed. Thereafter, the animals were sacrificed and the lungs were fixed by instillation of glutaraldehyde via the trachea under controlled hydrostatic pressure.[5]

Exposure Conditions

The four groups of animals received an intermittent inhalational exposure regimen of 8 hours/day and 5 days/week, except for public holidays and a 1-week rest following open lung biopsies. The animals were placed in open stainless steel cages, and the exposure took place in 7.5 m^3 capacity inhalational dust/pressure chambers. All test chambers featured controlled climatic conditions (25°C temperature, 70% relative humidity). One group of animals (quartz-exposed group) received a concentration of 5 mg/m^3 of DQ12 <5 µm (Dörentruper quartz) quartz-dust.[6] A second group (quartz/compressed air group) was exposed to a concentration of 5 mg/m^3 of DQ12 and additional hyperbaric conditions of 2.5 bar_a. A third group (compressed air group) was exposed to 2.5 bar_a only. A fourth group of animals (control group) was sham-exposed to clear normobaric air. The concentration of airborne respirable dust was measured with a TM digital µP photometer (OEB H. Hund GmbH, Wetzlar, FRG). The photometer reading was calibrated in terms of mass concentration of respirable dust by means of a gravimetric dust sampler.[7] In each test chamber, room temperature, humidity, pressure, and concentration of respirable dust were monitored and controlled continuously. Compression of the pressure chambers lasted 10–15 min. Decompression was initiated by a decompression step to 1.3 bar_a within 10 minutes, followed by decompression to 1.0 bar_a within 70 minutes.

Branchoalveolar Lavage

For BAL, the animals were anaesthetized with 15 mg/kg ketamine (Ketanest, Parke, Davis & Co., Munich, FRG) and

2 mg/kg xylacine (Rompun, Bayer, Leverkusen, FRG). With the animal in supine position, a flexible fiberoptic bronchoscope (BF P10, Olympus, Munich, FRG) was wedged into the main ronchus of the left lung. Following instillation of 100 ml of sterile 0.9% saline in aliquots of 20 ml, fluid was withdrawn, applying moderate suction. The lavage fluid was immediately filtered through sterile gauze, and the cells were pelleted at 300 g for 10 min. For some biochemical assays, the BAL supernate was examined in a fresh state. Otherwise, the supernate was aliquoted and stored at −70°C for further studies. In addition, possible bacterial contamination was assessed in each BAL sample. The BAL cells were washed twice and counted with a Coulter Counter. Cell viability was determined by the trypan blue exclusion technique. Cytocentrifuge smears served to identify the cellular populations stained with May-Grünwald-Giemsa, naphthyl acetate esterase and toluidine blue. Three hundred cells were counted, and the percentage of macrophages, lymphocytes, neutrophils, eosinophils and mast cells was determined.[8,9]

CT-Measurements

For CT-examinations, the animals were anaesthetized with 15 mg/kg ketamine and 2 mg/kg xylacine, and intubated. Immediately prior to each scan, the animals were hyperventilated to apnea and then scanned at a constant intratracheal pressure of 15 cm H_2O. CT-scans were performed in a Siemens Somatom DRH scanner (7s, 125 kV, 550 mAs). CT-sections were taken with a slice thickness of 1 mm at the level of the tracheal bifurcation, 5 cm cranially and 5 cm caudally, respectively. Lung areas of the thoracic scans were identified by using a modified ROI (region of interest)-method.[10] Starting from a manually specified line encircling each lung, contiguous pixels were analyzed. All pixels corresponding to chest wall, mediastinum and heart were eliminated based on their CT-numbers above −350 HU (Hounsfield units). Using histograms of CT-numbers, the mean CT-densities of both lungs were calculated in each animal.

Statistics

The results are expressed as means ± the standard error of the mean (x ± SEM). For data correlation, the Pearson correlation coefficient was calculated. The statistical comparison of group means was performed using One-Way Analysis of Variance and the Scheffé multiple comparison test. A p value <0.05 was considered to be statistically significant.

RESULTS

The exposure conditions were tolerated well by all animals. No signs of indisposition were observed during dust exposure, compression or decompression. The body weight of the animals kept stable and did not show any significant differences between the four test groups. However, during the entire observation period of 40 months, a total of seven animals was lost, due to severe fighting injuries or narcosis incidents respectively.

In all experimental groups, BAL cell viability and BAL fluid recovery did not change during the exposure period. However, total cell counts showed a bi-phasic profile, with a peak after 6 months followed by a decline and a final increase, starting after 18 months. The first peak of total cell counts was caused by increases of lymphocytes, macrophages, neutrophils and mast cells in that chronological order. In contrast, the final increase of total BAL cell counts was mainly due to a rise of neutrophils (Table I). Mean CT-densities were significantly ($p < 0.05$) augmented in both dust-exposed groups (Table II). There was perfect correlation of CT-densities, obtained after 27 months of exposure, and BAL total cell counts, obtained after 26 months ($r = 0.96$, $p < 0.001$).

DISCUSSION

The data obtained so far suggest that hyberbaric conditions do not accelerate or intensify the manifestation of silicosis dramatically. In contrary, particular data indicate some kind of a "protective" or delaying effect of long-term intermittent hyperbaric exposure. However, final conclusions may be drawn only when all measured parameters (cellular and biochemical BAL constituents, X-ray, CT-scan, various tests of lung mechanics and pulmonary gas exchange, morphometric pathohistology) will be evaluated and correlated. In our experimental model of silicosis, the serial BAL offered excellent insights into both the kinetics and function of free lung cells. In combination with biochemical factors of the BAL fluid (e.g., proteins, phospholipids, enzymes, immunological mediators, fibrogenic activity) more precise informations on the development of silicosis in a primate model will be available.

Under standardized conditions, the quantitative analysis of CT-scans proved to be a sensitive tool for the non-invasive assessment of structural pulmonary alterations. In addition, the perfect correlations of BAL-data and CT-data warrant more sophisticated analyses of CT-histograms as well as further studies on the validity of BAL in pneumoconioses.

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Table I

BAL Fluid Recovery, Total and Differential BAL Cell Counts Prior to and During Exposure

	months	control	quartz pressure	quartz	pressure
fluid recovery (%)	0	64.3 ± 3.9	65.4 ± 3.9	75.1 ± 1.6	74.4 ± 1.0
	6	70.5 ± 2.4	74.0 ± 1.8	74.1 ± 2.1	68.0 ± 3.0
	12	77.o ± 0.9	77.0 ± 1.1	75.7 ± 2.1	78.6 ± 1.0
	18	72.8 ± 5.6	76.6 ± 2.6	76.0 ± 0.9	77.7 ± 1.6
	24	79.4 ± 1.9	79.4 ± 1.4	80.5 ± 1.6	82.4 ± 2.3
total cell counts (x 10^6)	0	16.3 ± 2.2	15.1 ± 2.7	14.1 ± 2.9	14.1 ± 2.8
	6	19.9 ± 2.2	56.4 ± 8.6*	59.0 ± 5.0*	8.3 ± 1.2
	12	7.9 ± 1.1	26.2 ± 4.4*	37.7 ± 3.2*	5.5 ± 0.7
	18	9.6 ± 2.4	49.3 ± 6.2*	34.4 ± 4.0*	6.5 ± 1.4
	24	14.1 ± 3.5	82.6 ± 11.1*	56.0 ± 6.0*	4.2 ± 1.1
macrophages (%)	0	87.2 ± 3.2	84.0 ± 2.7	93.3 ± 1.9	88.7 ± 3.9
	6	84.0 ± 2.6	74.4 ± 4.2	76.1 ± 2.9	87.6 ± 3.6
	12	85.8 ± 3.0	74.3 ± 2.6	77.8 ± 2.8	82.7 ± 4.4
	18	85.6 ± 4.2	68.3 ± 1.3*	74.8 ± 3.9	85.2 ± 3.6
	24	82.6 ± 6.4	65.0 ± 3.3	65.8 ± 4.3	86.0 ± 3.3
lymphocytes (%)	0	6.3 ± 2.0	5.1 ± 0.6	2.7 ± 0.8	4.1 ± 0.9
	6	9.3 ± 1.8	9.6 ± 1.4	8.3 ± 0.9	5.0 ± 1.0
	12	6.2 ± 1.9	9.1 ± 1.8	4.5 ± 1.2	6.6 ± 1.7
	18	7.2 ± 2.4	7.4 ± 1.3	5.4 ± 0.5	5.7 ± 1.4
	24	8.4 ± 2.0	10.9 ± 1.4	11.8 ± 2.3	6.2 ± 1.4
neutrophils (%)	0	2.5 ± 1.1	4.0 ± 1.7	2.1 ± 1.7	1.0 ± 0.4
	6	1.3 ± 0.5	6.4 ± 1.1	8.3 ± 2.7	0.4 ± 0.3
	12	0.7 ± 0.5	7.7 ± 1.3	9.0 ± 3.9*	0.1 ± 0.1
	18	1.0 ± 0.5	11.3 ± 1.6*	11.1 ± 4.0*	0.3 ± 0.2
	24	2.6 ± 1.5	16.0 ± 3.1*	9.5 ± 1.6	0.2 ± 0.2
mast cells (%)	0	1.8 ± 0.9	1.3 ± 0.7	1.0 ± 0.7	1.3 ± 1.1
	6	2.8 ± 1.5	3.7 ± 1.7	5.6 ± 1.2	3.1 ± 1.8
	12	5.3 ± 1.9	6.6 ± 2.1	6.0 ± 1.7	4.9 ± 1.5
	18	4.0 ± 1.4	11.9 ± 1.6*	7.8 ± 2.0	4.0 ± 1.8
	24	5.4 ± 2.8	7.6 ± 2.3	11.8 ± 3.8	4.2 ± 1.2

Data expressed as mean ± SEM.
* $p < 0.05$ vs. control

Table II

BAL Fluid Recovery, Total and Differential BAL Cell Counts, and Mean CT-Densities after 26 Months of Exposure.

	control (n = 5)	quartz (n = 7)	quartz pressure (n = 4)	pressure (n = 5)
fluid recovery (%)	79.8 ± 1.8	80.7 ± 1.4	80.8 ± 2.3	80.2 ± 1.4
total cell counts (x 10^6)	7.0 ± 0.9	79.6 ± 20.3*	61.4 ± 2.3	5.1 ± 1.8§
macrophages (%)	87.4 ± 4.4	69.8 ± 5.7*	78.5 ± 2.2	88.0 ± 1.6§
lymphocytes (%)	4.2 ± 1.6	5.5 ± 1.3	3.8 ± 0.8	5.4 ± 1.7
neutrophils (%)	0.6 ± 0.4	19.5 ± 7.6*	10.5 ± 2.7	0.2 ± 0.2
mast cells (%)	4.6 ± 2.9	5.3 ± 1.9	7.3 ± 2.9	2.4 ± 1.4
mean CT-density (HU)	-892.3 ± 8.5	-734.6 ± 27.5*	-800.0 ± 7.5	-908.6 ± 7.8§

Data expressed as mean ± SEM.
* $p < 0.05$ vs. control group
§ $p < 0.05$ vs. quartz-exposed group
$ $p < 0.05$ vs. quartz/pressure-exposed group

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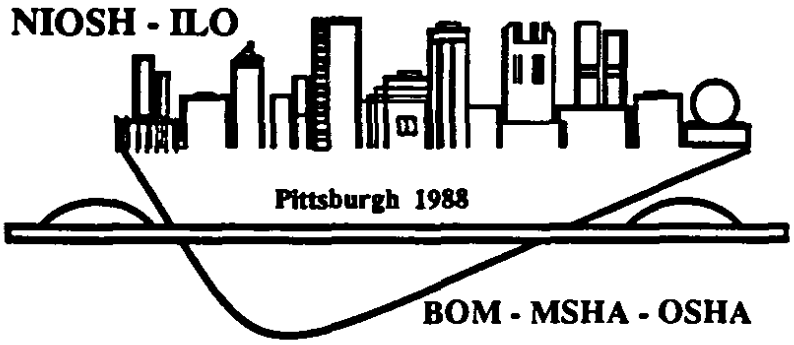

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