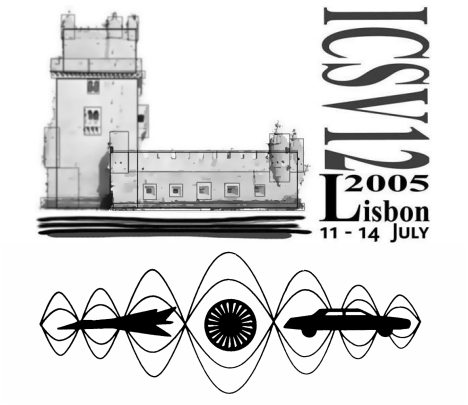

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MUTAGENESIS AND MALIGNANCY IN VIBROACOUSTIC DISEASE

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Abstract

Introduction. The notion that low frequency noise (LFN, <500 Hz, including infrasound) could be associated with mutagenesis was not initially obvious. However, over the past 25 years of research into the effects of LFN exposure, the genotoxic component of LFN became undeniable. The goal of this study is to present an overview of what is known to date on malignancy associated with Vibroacoustic Disease (VAD). Methods. A chronological overview of the mutagenic evidence of LFN exposure is undertaken. Past studies on the frequency of sister chromatid exchanges (a widely used genotoxic assay) in LFN-exposed workers and rodents are described. Metaplastic and dysplastic appearances, and cellular de-differentiation seen in LFN-exposed animals are presented. Description of malignancy findings among LFN-exposed workers is also taken into account. Results. The large amount of malignant tumours identified among a population of aircraft technicians, many of them multiple and often of silent evolution, was the first indication that LFN might be a genotoxic agent. Studies on the frequency of sister chromatid exchanges showed an increase in LFN-exposed workers and in LFN-exposed animal models, thus confirming LFN as genotoxic agent. In extensive studies of the respiratory tract of LFN-exposed rodents, abnormal cellular organization of epithelial tissues, normally considered to be pre-cursors to the formation of tumours, was consistently observed in the trachea and lungs. All (100%) respiratory tract cancers in LFN-exposed workers diagnosed with VAD were, precisely, squamous cell carcinomas. Concurrently, the vast majority of the tumours identified in VAD patients were located in hollow organs. Discussion. The issue of an acoustical stressor being a cancer-causing agent is still in its infancy. The evidence gathered to date indicates that much more urgent attention needs to be given to LFN as a genotoxic agent.

INTRODUCTION

The neurological abnormalities in aircraft technicians began to be studied by a multidisciplinary team, headed by the author, in the early 1980's. Until 1987, a multitude of information had been gathered on these patients' brainstem auditory evoked potentials (1), P300 evoked response potentials (2,3), brain MRI (1) and neurological examination (4)[1]. Some of these workers, exposed to high levels of low frequency noise (LFN, ≤500 Hz, including infrasound), developed late-onset epilepsy (5), and balance disorders (2)[1], while many exhibited the palmo-mental reflex (6) and, in a few, auditory stimuli elicited facial dyskinesia (7)[1].

Today, the signs and symptoms developed by individuals excessively exposed to LFN is called vibroacoustic disease (VAD) (8,9). The clinical stages of VAD, as determined for a group of aircraft technicians is given in Table 1.

Table 1 - Data from a group of 140 aircraft technicians. LFN exposure time (years) refers to the amount of time it took for 70 individuals (50%) to develop the corresponding sign or symptom (9).

Clinical Stage	Sign/Symptom
Stage I-Mild (1-4 years)	Slight mood swings, Indigestion & heart-burn, Mouth/throat infections, Bronchitis
Stage II-Moderate (4-10 years)	Chest pain, Definite mood swings, Back pain, Fatigue, Fungal, viral and parasitic skin infections, Inflammation of stomach lining, Pain and blood in urine, Conjunctivitis, Allergies
Stage III–Severe (> 10 years)	Psychiatric disturbances, Haemorrhages of nasal, digestive and conjunctive mucosa, Varicose veins and haemorrhoids, Duodenal ulcers, Spastic colitis, Decrease in visual acuity, Headaches, Severe joint pain, Intense muscular pain, Neurological disturbances

Table 1 refers to the signs and symptoms developed specifically by aircraft technicians working the standard 8 hrs/day, 5 days/week. Not all LFN-exposed workers have this exposure schedule. For example, ship machinists can spend 3 weeks onboard ship (i.e., exposed to substantial LFN-rich environments) and 2 weeks at home (i.e., presumably not in LFN-rich environments) (10). Other professional activities exist where the LFN-exposure time pattern is not the standard 8-hour/day exposure, such as with submarine and oil rig operators, and astronauts. In these cases, the evolution of signs and symptoms could be greatly accelerated. Moreover, since different LFN environments have unique frequency distributions, the fact that some frequency bands may be more predominant than others (i.e., concentrate more acoustical energy) can lead to the development of slightly different pathology. If the LFN exposure is environmental and/or leisurely, the standard 8hr/day model is also not applicable.

[1] See "Neurological disorders in vibroacoustic disease I and II," included in these Proceedings.

Until 1987, it was thought that the pathology observed in aircraft technicians was solely of a neuro-psychological and –physiological nature. However, in that year, the first autopsy of a VAD patient was conducted (11). This technician worked for the Portuguese Navy for 10 years as a ship machinist. In 1959 he was hired as an aircraft engine technician, where he worked for another 26 years, performing aircraft run-up tests on a daily basis. Diagnosed with late-onset epilepsy in 1981, this man died at age of 58, of cardiac tamponade caused by a small infarct. His heart disclosed 11 small scars of previous silent ischemic events. Cardiac valves seemed swollen, and the pericardium surrounding the heart was greatly thickened. Coronary arteries were thickened, but not by the usual, and expected, artherosclerotic plaques. Instead, a continuous thickening of the intima lined all vessel walls. Locations where artherosclerotic plaques would be expected exhibited thicker walls Microscopic studies later revealed that much of the thickening was due to abnormal proliferation of collagen fibers. Two (previously silent) tumours were found, a Grawitz in the kidney, and a grade I, microcystic astrocytoma in the right parietal region of the brain. Both lungs disclosed interstitial and focal fibrosis.

The goal of this report is to put forth what is known on the genotoxic and mutagenic effects of LFN exposure, as gathered within the scope of VAD studies.

TUMOURS IN AIRCRAFT TECHNICIANS

In 1996, the medical and clinical histories of 236 aeronautical workers (ave. age 43 yr, SD=6.4) were surveyed, and compared with those of 964 workers (ave. age 46 yr, SD=8.5), employed by the same aeronautical plant, but that did not work in LFN-rich environments. In the control population, there were 8 cases of malignancy. In the LFN-exposed workers, there were 28, i.e., 11%, although the occurrence of tumours was insufficient to warrant inclusion in Table 1 (12). Five of these 28 cases were instances of multiple different tumours, not related to metastatic events, and not necessarily simultaneous. A large number of tumours were found within hollow organs, such as lung, bladder and stomach. All 5 cases of brain tumours were malignant glial-type. There was 1 case of soft tissue tumour. Post-operatory prognosis in these patients is very good, with very few cases of reincidence.

Single type of Tumour in Lung

In this group of 236 workers, 5 cases developed malignant lung carcinomas lesions, 2 in non-smokers. All were squamous cell carcinomas, and aspects of low-grade differentiation of these tumours are also distinctive.

To date, 10 male VAD patients (ave. age: 50 $\pm$ 5 years, 3 non-smokers) have developed respiratory tract tumours (13). *All* have been poorly differentiated squamous cell carcinomas, 2 located in the glottis and 8 in the upper right lobe of the lung. Of the 3 non-smokers, 2 had lung tumours and 1 had a glottis tumour. The 2 surviving patients are both heavy smokers.

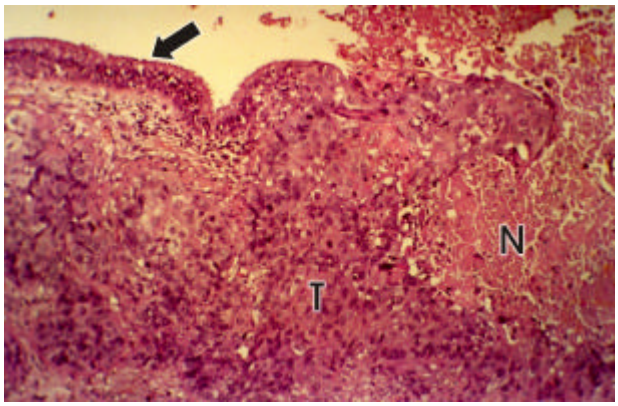

Figure 1- (Light microscopy x100) Bronchial tumour. From left to right: bronchial mucosa is almost normal with evident cilia (arrow); transition to tumour (T) and then necrotic tumour (N). Deep in the bronchial wall is the structure of a squamous cell carcinoma (N).

Epidemiological studies indicate that squamous cell carcinomas account for approximately 40% of all lung tumours in men (14). However, LFN exposure was not taken into account, and thus it is unknown how many of these could be related to LFN exposure.

All VAD patient lung tumours (N=8) have been located in the upper right lobe. This might appear to be trivial, but these results are corroborated by an experiment conducted by Ponomarkov *et al.* in 1969 (15). Here the authors explored the effects of wide-band noise at 105-155 dB on dogs . After 1.5-2 hours of exposure, the animals were sacrificed. Autopsy results revealed 3mm diameter haemorrhages in the lungs of the animals exposed to about 126 dB, and which were located under the pleura. These haemorrhages were most commonly found in the costal surface of the upper lobe of the right lung. There may be a biomechanical explanation for this feature: because of the position of the heart relative to the left lobe, the acoustical impedance and resonance of the left lobe will most probably be different than that of the right lobe. The difference in the acoustical properties inherent to different organ geometry most probably plays an important role in the location of the development of lung tumours in VAD patients (and haemorrhages in noise-exposed dogs), and may partially explain the apparent increased susceptibility of the right lobe.

ANIMAL MODELS

In 1992, given the atypical cases of respiratory pathology seen in VAD patients, Wistar rats were exposed to LFN for detailed studies of pleural immune responses (16,17)[2]. Several exposure schedules were used and, with time, second and third generation rats were born within LFN-rich environments. Figure 2 shows the teratogenic consequences of exposure to this type of stressor.

[2] See "Respiratory pathology in vibroacoustic disease I and II," included in these Proceedings.

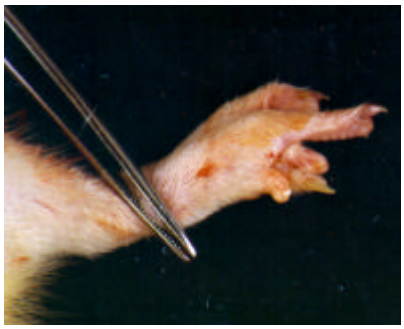

Figure 2 - Limb of a third generation rat born within a LFN environment with evident gross malformations that include loss of segments (15).

Concurrently, respiratory tract epithelia was being studied as to the LFN-induced morphological changes of cellular and tissue structures (18,19)[2]. Among the plethora of information gathered through these LFN animal models was metaplastic and dysplastic cellular organizations, considered an early precursor to cancerous lesions (see Figs. 3,4). No tumours were ever identified in the rats, perhaps because rat life span is too short for tumour development, given this particular type of acoustic stimulus.

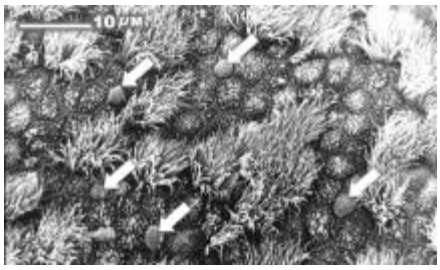

Figure 4 - Scanning electron microscopy of rat tracheal epithelium (gestated and born in LFN, and then exposed to 2213 hours of occupationally-simulated LFN). The amount of cilia is greatly reduced. Rosetta structures are visible, but SC are irregularly shaped. BC (arrows) are distinctly visible in the centre of the rosettas. BC microvilli have lost their uniform distribution, and SC microvilli are mostly short and stubby. Intercellular junctions are thick and prominent, and SC surfaces are flat or slightly sunken. (18,19)

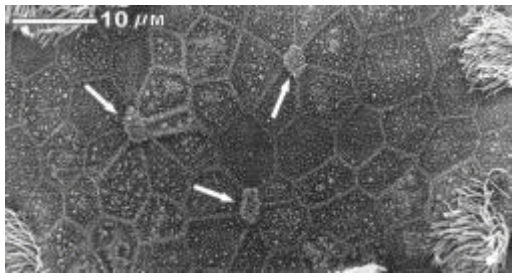

Figure 3 – Scanning electron microscopy of control rat tracheal epithelium. All arrows point to brush cells (BC). Several BC have tufted microvilli, & are surrounded by a ring of secretory cells (SC) in rosetta-shaped formations. SC microvilli are in different stages of development, i.e., at different life cycles. BC & SC microvilli sprout uniformly with same density and shape in all the cells.. Exuberant cilia, are also present. (18,19)

GENOTOXIC EFFECTS OF LOW FREQUENCY NOISE EXPOSURE

Sister chromatic exchange is a symmetrical exchange of chromatid segments within a single chromosome that does not result in an overall structural change in the chromosome. The points of exchange in the two sister chromatids appear to occur cytologically at the same locus, but the precision of exchange at the molecular level is not known. The exact mechanism of SCE formation is not clear. It appears that SCE production is dependent on DNA synthesis (20) and may be related to DNA breakage or to misrepair of DNA lesions (21). Althoug
h there are uncertainties regarding the mechanisms of formation, SCE analysis is one of the most sensitive genotoxic assays and has been widely used for detecting human exposure to potentially genotoxic agents (22).

Frequency of SCE was investigated in a group of 50 workers, employed at different workstations within the same plant: Group 1 (G1) consisted of 10 operators of hand vibrating tools (riveting machines and pneumatic hammers), exposed to local vibration and LFN (23). Groups 2 and 3 consisted of aircraft technicians working as engine test-cell operators (G2, N = 15) and aircraft run-ups (G3, N = 12). Group 4 (G4) consisted of 13 helicopter pilots. The control group (G0) consisted of 34 unexposed male office workers also employed at the same plant. Highly significant differences were found between aircraft technicians and helicopter pilots (G2, G3, G4) and controls (G0). No significant differences were found between aircraft technicians and helicopter pilots (G2, G3, G4), nor between pneumatic hammer operators (G1) and controls (G0). Group differences were essentially unaffected when analysis was confined to smokers only or to non-smokers only, and no age-related effects were detected (23).

These studies were later extended to another LFN-exposed professional group, military aircraft pilots (24), as well as to the LFN-exposed animal models (25). All provided evidence that LFN was, indeed, a genotoxic agent.

CONCLUSIONS

The studies contained herein have a wide variety of implications. Foremost is that despite being a genotoxic agent, LFN is not considered a variable when cancer epidemiology studies are conducted. Thus, the vast majority of cancer studies do not include patients' LFN exposure histories, and consequently, do not take into account the genotoxicity of LFN.

Another noteworthy feature is the appearance of a single type of histological tumour in VAD patients with respiratory tract cancer (squamous cell carcinomas). Simultaneously, metaplasia and dysplasia were seen in LFN-exposed rats.

Multiple silent tumours in VAD patients are not uncommon and must be taken into consideration by medical professionals who have noise-exposed workers under their care.

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