

NOISE-INDUCED GASTRIC LESIONS: A LIGHT AND SCANNING ELECTRON MICROSCOPY STUDY OF THE ALTERATIONS OF THE RAT GASTRIC MUCOSA INDUCED BY LOW FREQUENCY NOISE

Jorge da Fonseca¹, José Martins dos Santos¹, Nuno Castelo Branco², Mariana Alves-Pereira³, Nuno Grande⁴,
Pedro Oliveira¹, Ana Paula Martins⁵

¹Egas Moniz Institute of Health Sciences, Caparica, Portugal

²Center of Human Performance, Alverca, Portugal

³Department of Environmental Sciences and Engineering, New University of Lisbon, Caparica, Portugal

⁴Abel Salazar Biomedical Sciences Institute and Multidisciplinary Unit of Biomedical Investigation, University of Porto, Porto, Portugal

⁵Department of Pathology, Hospital of Santa Cruz, Carnaxide, Portugal

SUMMARY

Introduction: Exposure to low frequency noise (LFN) can lead to vibroacoustic disease (VAD), recognized as a systemic disease with lesions in a broad spectrum of organs. Although gastrointestinal complaints are common among individuals exposed to noise, only few studies tried to evaluate the digestive lesions. The authors performed this study in order to investigate gastric lesions in an animal model of VAD.

Material and methods: Adult Wistar rats were exposed to continuous LFN. After five weeks they were sacrificed. The stomachs were studied by light microscopy and scanning electron microscopy, and compared with stomachs of animals kept in silence.

Results: Superficial erosions were present in the noise-exposed animals. Massive cell death of the gastric epithelium was observed, both by light and electron microscopy.

Discussion: The erosions, reflecting cellular degeneration and death, occurred without inflammation, similar to what has been observed in other LFN-exposed organs.

Key words: gastric cell death, gastric erosions, light microscopy, scanning electron microscopy, low frequency noise, vibroacoustic disease, wistar rat model

Address for correspondence: Jorge da Fonseca, Passeio dos Mastros, Lote 4.17.01.L – 2º C, Jardins Expo – 1990, Lisboa, Portugal.
E-mail: jorgedafonseca@hotmail.com

INTRODUCTION

Vibroacoustic disease (VAD) is a systemic disease caused by long-term exposure to low frequency noise (LFN, <200 Hz, including infrasound, <20 Hz). VAD has been associated with occupational exposure to LFN at the workplace (1), namely in aircraft engine technicians. VAD-related lesions have been described in several organs. Previous studies on VAD have reported lesions in the nervous system (2, 3, 4), heart (5, 6), blood vessels (7), lymphatics (8) and respiratory tissues (9). LFN and vibration can influence the stomach and intestine functions (10). Since the early reported human cases of VAD, gastro-intestinal (GI) complaints are documented in this disease (11, 12, 13). Clinically, GI complaints usually begin after 3 years of professional activity involving exposure to LFN (11). Malignant digestive tumours of the stomach, colon and rectum seem to occur with increased frequency in LFN-exposed patients (13). Although gastrointestinal (GI) complaints are a common occurrence among individuals exposed to noise, there are not many studies aiming at the investigation of the effects of LFN on the GI tract. The present study intends to be a contribution to the evaluation of digestive LFN-induced lesions.

A few studies have aimed at the investigation of the extra-auditory effects of acoustic stimuli on the GI tract, reporting effects of noise within a large frequency range. Anthony (14) reported stomach constrictions in noise-exposed guinea pigs when at the limits of acoustic tolerance for the animals, at 144 dB ranging from 300 to 4800 Hz frequency range. Kim et al. (15) studied the alterations of gastric acid output in healthy volunteers and dogs exposed to the noise of a jet engine. They also found ulcerative gastric lesions in rats exposed to this kind of noise. Cohen (16) reported stomach cramps, nausea, diarrhoea, and heartburn as frequent complaints among boiler-plant workers. He observed that the number of these complaints increased despite the implementation of a hearing protection program inside the plant. Cohen found no explanation to this increase of the number of digestive disorders. We assume that the use of a hearing protection might induce a less protective attitude and increase the workers exposure to digestive extra-auditory effects of noise.

Although whole-body vibration (WBV) and LFN are similar physical phenomena, their propagation media before impinging on the biological tissues are distinct. Nevertheless, the results obtained with human experience and animal models exposed to WBV may provide insight into the lesions observed in the LFN-

exposed, VAD animal model. Some reports have evaluated the human gastric motility in mechanically induced WBV (17, 18, 19). Bosco (20) studied the alterations of the neural and hormonal responses in WBV-exposed men. Nakamura et al. (21), in an elegant experimental study, found WBV-induced lesions in the rat model. The authors addressed the relationship between the alterations of the neural and hormonal responses in WBV and the lesions of the stomach. They compared the effects of vibration with those of forced water immersion (FWI) of the animals, a known cause of stress in rats. They found that both mechanisms produced alterations on gastric blood flow, on plasma corticosterone and catecholamines, leading to the formation of mucosal erosions and ulcerations. Truncal vagotomy partially decreased FWI alterations, but not those caused by vibration, showing that the vibration-induced gastric lesions were not entirely mediated by the nervous system, and indicating a specific role for a direct mechanical effect of vibration on the stomach. Although the gastric lesions were based on a macroscopic evaluation of the superficial mucosal layer, the authors found a large number of gastric erosions in WBV-exposed animals.

LFN is present in a large variety of every day life activities, from the disco clubs to factory environments or urban traffic noise. Given the ubiquitous nature of LFN in modern society, the study of GI complaints associated with noise exposure among human populations is a daunting task. However, within the scope of VAD studies, animal models exposed to well-defined sources of LFN have been in use since 1992 and have provided important insights on the interactions of acoustic phenomena and biological tissues, namely with regards to respiratory structures (22, 23, 24, 25).

The paucity of comprehensive studies on noise-induced digestive pathology is striking, considering the number of reports suggesting GI complaints in association with noise exposure. There are no previous experimental studies on the morphological alterations of gastric mucosa induced by LFN exposure. Therefore, the goal of the present study was to investigate the morphological injury of LFN-exposed gastric tissue of the Wistar rat, as an experimental animal model of VAD, using light and SEM microscopy.

MATERIAL AND METHODS

Animals

Thirty adult Wistar rats were divided in two groups of 15 animals: Noise-exposed group (6 males and 9 females – mean weight: 248 ± 35 g) was exposed 24 hours per day for 5 weeks to LFN (850 hours). Control group (7 males and 8 females – mean weight: 253 ± 42 g) was kept in silence in the animal facility. The animals were housed in groups of 2 or 3 rats per cage, in which they could move around. They had light cycles of 12 hours day/12 hours night. All animals were fed with standard rat food, had unrestrained access to water, and were treated according with the EU directive on Animal Protection for Experimental and Scientific Purposes (86/609/EEC).

Low Frequency Noise Exposure – Experimental Protocol

A noise generator produced an amplified and frequency-filtered signal, creating an acoustic environment rich in low frequency components. Figure 1 shows the overall linear (dBL)

noise level and the spectral analysis, analysed by a digital real time analyser, near the position of the LFN-exposed rats. Levels were above 90 dB in the frequencies ranging from 50 to 200 Hz.

Microscopy

Rats were sacrificed with a lethal intraperitoneal injection of pentobarbital. According to the method described by Nakamura et al. (1992), stomach was removed. After removing a small fragment for electron microscopy, the cardiac end was ligated and 10 ml of 1% formalin was introduced from the duodenal end, which was then ligated. After 30 min of exposure to formalin, the stomach was opened along the greater curvature. After macroscopic observation, the specimen was fixed in 10% buffered formalin for light microscopy (LM). Sections were stained with haematoxylin-eosin and Masson trichromic solution for LM observation.

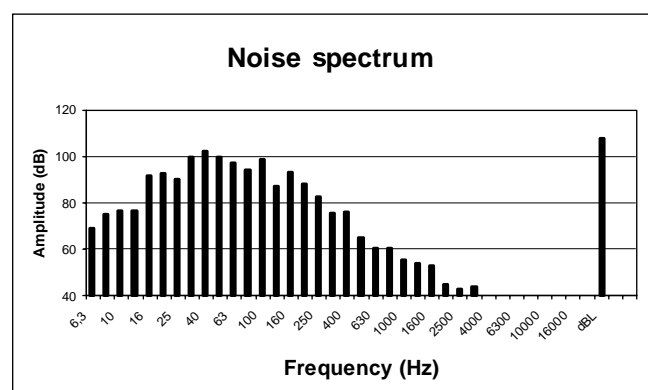


Fig. 1. Frequency distribution of the acoustic environment to which the rats were exposed.

For scanning electron microscopy (SEM), the specimens were washed in phosphate buffer saline (PBS), fixed at room temperature in an aldehyde mixture made up of 4% formaldehyde, 1.25% glutaraldehyde and 10 nmol/l CaCl_2 in 0.05 mol/l cacodylate buffer. For scanning electron microscopy (SEM) the samples were dehydrated in ethanol and critical point-dried in a Balzer's apparatus using carbon dioxide as the transitional fluid. The preparations were mounted on metal stubs with conductive carbon paste. The specimens were coated with Au/Pt under vacuum and examined in a Jeol (Japan) JSM-35C scanning electron microscope. The instrumental conditions for the observations were 15 kW, 0.2 nA, 30 s/frame (photography).

RESULTS

Control rats had slight weight increase of 23 g (mean weight from 253 g to 276 g). The stomachs of control rats had a normal macroscopic appearance and they had a normal glandular epithelial layer (Fig. 3a) even though, observed with SEM, the normal glandular epithelial surface showed sparse images of dying cells (Fig. 4a).

Noise-exposed rats had slight weight decrease of 12 g (mean weight from 248 g to 236 g). Regardless of the gender, stomachs showed morphological alterations. Macroscopically, some superfi-

cial erosions were present, mostly in the glandular distal part of the stomach (Fig. 2).

LM alterations were seen in the glandular epithelial layer of the distal region of the stomach, present in all noise-exposed stomachs. There was evidence of cellular distress and degeneration, with picnosis and massive cell death (Fig. 3b). There were no signs of acute or chronic inflammatory infiltrate. Lesions of

variable severity were observed over the entire pyloric region. The mucous layer presented irregular diffuse impairment.

With SEM, the surface of the epithelial layer of the stomachs in controls was observed and seldom showed evidence of dying cells (Fig. 4a). In contrast, in noise-exposed stomachs, massive cell death was observed over the entire distal surface of the epithelium (Fig. 4b).

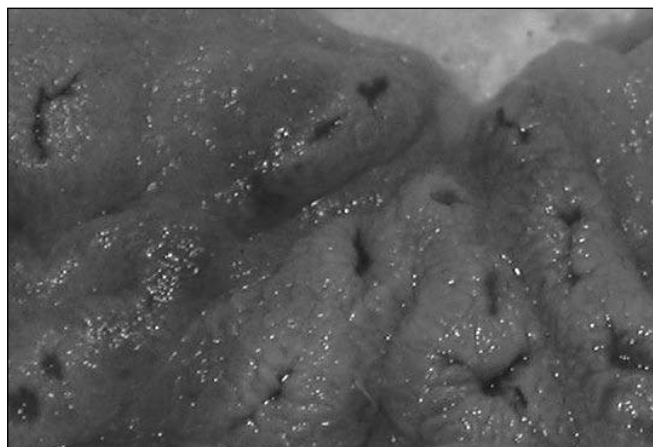


Fig. 2. Macroscopic images of gastric erosions in a noise-exposed stomach.

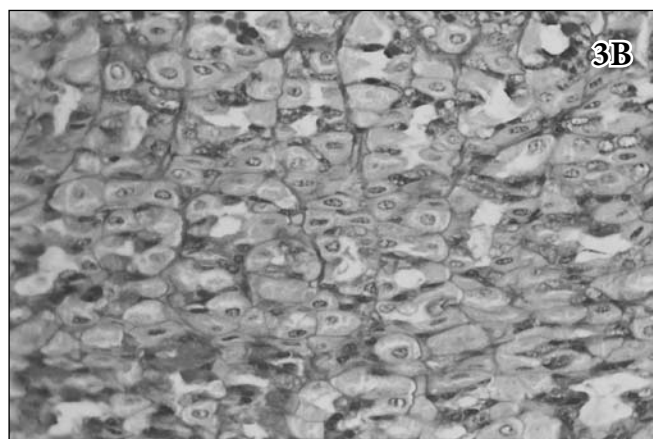
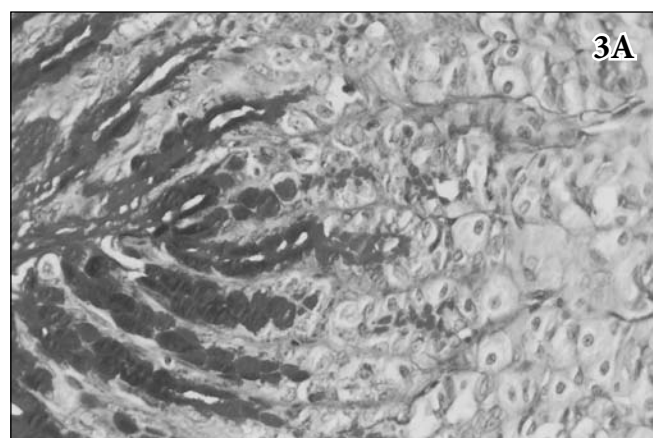


Fig. 3. Light micrograph of epithelial layer of rat stomach (hematoxylin-eosin $\times 200$). Normal aspects from control rat (Fig. 3a) and degeneration and cell death of the glandular epithelial layer of noise-exposed stomach (Fig. 3b).

DISCUSSION

Nakamura et al. (21) found gastric erosions in WBV-exposed rats and proposed a direct mechanical effect of vibration as a pathogenic mechanism of lesions. Although based only on

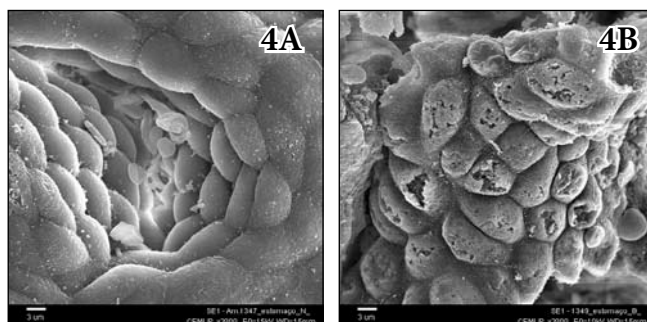


Fig. 4. SEM micrographs of epithelial layer of rat stomach ($\times 2,000$). Normal aspects from control rat (Fig. 4a) and massive cell death observed in noise-exposed rat (Fig. 4b).

a macroscopic evaluation, without any microscopic characterization of these lesions, they proved the usefulness of the rat experimental model of WBV (21). Our study demonstrated the usefulness of the Wistar rat model to study LFN-induced alterations of gastric mucosa.

Lesions were identified in the stomachs of all LFN-exposed rats. Two features were most striking: i) increased frequency of cell death in the glandular epithelial layer of the distal stomach, by both LM and SEM, and ii) erosions which were observed both macroscopically and by LM, reflecting cellular degeneration and death, seen by LM, as well as in the widespread death of epithelial cells documented by SEM.

These alterations occurred without inflammation, which is consistent with what has been observed in other LFN-exposed organs such as in the lung (9), in the pericardium and cardiac valves (5, 6) and in vascular structures, both in humans (26) and animal models (7). Ulcerous and erosive lesions are known to be related with some of the digestive complaints that are referred by VAD patients. The mechanism of the lesions is unknown.

Both neurological and endocrine mediations are possible.

The stress of the experimental conditions may also be important. Given the experience of other tissues within the VAD spectrum and of gastric lesions in WBV, a direct effect of noise and vibration on the stomach wall is plausible.

As shown by our data, in our experimental conditions, the stomach of rat is sensitive to LFN-induced lesions.

The herein study encourages further investigations to define the pathophysiology and evolution of gastric lesions among LFN-exposed individuals and also in VAD patients.

REFERENCES

- Castelo Branco NA, Rodriguez E. The vibroacoustic disease - an emerging pathology. *Aviat Space Environ Med.* 1999;70(3 Pt 2):A1-6.
- Martinho Pimenta A, Castelo Branco NA, Manaças JE. Neurologic study in 45 subjects exposed to noise and industrial vibration. *Med Aeronaut Spat.* 1991;119:359-62. (In French.)
- Maurício JC, Branco G, Pimenta AJ, Castelo Branco MS, Castelo Branco NA. Noise and vibration exposure effects on C.N.S. M.R.I. Study. *Med Aeronaut Spat.* 1991;119:363-7. (In French.)
- Martinho Pimenta AJ, Castelo Branco NA. Neurological aspects of vibroacoustic disease. *Aviat Space Environ Med.* 1999;70(3 Pt 2):A91-5.
- Castelo Branco NA, Aguas AP, Sousa Pereira A, Monteiro E, Fragata JI, Tavares F, Grande NR. The human pericardium in vibroacoustic disease. *Aviat Space Environ Med.* 1999;70(3 suppl.):A54-62.
- Marciniak W, Rodriguez E, Olszowska K, Atkov O, Botvin I, Araujo A, Pais F, Soares Ribeiro C, Bordalo A, Loureiro J, Prazeres De Sa E, Ferreira D, Castelo Branco MS, Castelo Branco NA. Echocardiographic evaluation in 485 aeronautical workers exposed to different noise environments. *Aviat Space Environ Med.* 1999;70(3 suppl.):A46-51.
- Martins dos Santos J, Grande NR, Castelo Branco NA, Zagalo C, Oliveira P. Vascular lesions and vibroacoustic disease. *Eur J Anat.* 2002; 6(1):17-21.
- Martins dos Santos J, Grande NR, Castelo Branco NA, Zagalo C, Oliveira P, Alves-Pereira MM. Lymphatic lesions and vibroacoustic disease. *Eur J Lymphol.* 2004;12(40):17-20.
- Reis Ferreira JM, Couto AR, Jalles-Tavares N, Castelo Branco MS, Castelo Branco NA. Airway flow limitation in patients with vibroacoustic disease. *Aviat Space Environ Med.* 1999;70(3 Pt 2):A63-9.
- Maschke C. Introduction to the special issue on low frequency noise. *Noise Health.* 2004;6(23):1-2.
- Castelo Branco NA. The clinical stages of vibroacoustic disease. *Aviat Space Environ Med.* 1999;70(3 suppl.):A32-9.
- Castelo Branco NA. A unique case of vibroacoustic disease: a tribute to an extraordinary patient. *Aviat Space Environ Med.* 1999;70(3 suppl.):A27-31.
- Castelo Branco NA, Alves-Pereira M. Vibroacoustic Disease. *Noise Health.* 2004;6(23):3-20.
- Anthony A, Harclerode JE. Noise stress in laboratory rodents. II. Effects of chronic noise exposure on sexual performance and reproductive function of guinea pigs. *J Acoust Soc Am.* 1959;31(11):1437-40.
- Kim CY, Ryu JS, Hong SS. Effect of air-craft noise on gastric function. *Yonsei Med J.* 1968;9(2):149-54.
- Cohen A. The influence of a company hearing conservation program on extra-auditory problems in workers. *J Safety Res.* 1976;8(4):146-62.
- Kjelberg A, Wikstrom BO. Acute effects of whole-body vibration. Stabilography and electrogastrigraphy. *Scand J Work Environ Health.* 1987;13(3):243-6.
- Ishitake T, Kano M, Miyazaki Y, Ando H, Tsutsumi A, Matoba T. Whole-body vibration suppresses gastric motility in healthy men. *Ind Health.* 1998;36(6):93-7.
- Ishitake T, Miyazaki Y, Ando H, Matoba T. Suppressive mechanism of gastric motility by whole-body vibration. *Int Arch Occup Environ Health.* 1999;72(7):469-74.
- Bosco C, Iacovelli M, Tsarpela O, Cardinale M, Bonifazi M, Tihanyi J, Viru M, De Lorenzo A, Viru A. Hormonal responses to whole-body vibration in men. *Eur J Appl Physiol.* 2000;81(6):449-54.
- Nakamura H, Katoh A, Nohara S, Nakamura H, Okada A. Experimental studies on the pathogenesis of the gastric mucosal lesions induced by whole-body vibration. *Environ Res.* 1992;58(2):220-9.
- Grande NR, Aguas AP, Sousa Pereira A, Monteiro E, Castelo Branco NA. Morphological changes in rat lung parenchyma exposed to low frequency noise. *Aviat Space Environ Med.* 1999;70(3 suppl.):A70-7.
- Sousa Pereira A, Grande NR, Monteiro E, Castelo Branco NA. Morphofunctional study of the rat pleural mesothelial cells exposed to low frequency noise. *Aviat Space Environ Med.* 1999;70(3 suppl.):A78-85.
- Oliveira MJ, Pereira AS, Guimaraes L, Freitas D, Carvalho AP, Grande NR, Aguas AP. Chronic exposure of rats to cotton-mill-room noise changes the cell composition of the tracheal epithelium. *J Occup Environ Med.* 2002;44(12):1135-42.
- Castelo Branco NA, Alves-Pereira M, Martins dos Santos J, Monteiro E. SEM and TEM study of rat respiratory epithelia exposed to low frequency noise. In: Mendez-Vilas A, editor. *Science and technology education in microscopy: an overview.* Vol II. Badajoz (Spain): Formatex; 2003. p. 505-33.
- Albuquerque e Sousa J, Dinis da Gama A, Macedo MV, Cassio I, Pimenta FM, Castelo Branco NA. Carotid angiodynographic studies in individuals with the whole-body noise and vibration disease. *Revista Portuguesa de Medicina Militar.* 1991;39:63-5.

*Received August 29, 2005
Received in revised form and accepted
November 4, 2005*