Iranian Biomedical Journal 12 (2): 129-132 (April 2008)

Short Report

Morphological Changes of Rabbit Lacrimal Gland Cells from Amiodarone Administration

Fereshteh Mehraein

Histology Dept., Medical School, Iran University of Medical Sciences, Hemmat Highway, Tehran, P.O. Box 1449614525, Iran

Received 11 March 2007; revised 22 September 2007; accepted 14 October 2007

ABSTRACT

Background: Amiodarone is a drug that is used for treatment of cardiac arrhythmia after cardiac ischemia. This drug as β blocker decreases arrhythmia rate but it has many side effects on different tissues. Since there are rare reports about changes of lacrimal glands, this research has been carried out to study the morphological and ultrastructural changes of lacrimal gland cells after amiodarone administration. **Methods**: Male rabbits (n = 14) were divided into control and experimental groups. Experimental group were intra peritoneally injected with a daily single dose of 80 mg/kg amiodarone for two weeks. The control group only received normal saline. At the end of the injection period, the two groups were anesthetized and perfused with Karnovsky's fixative. The lacrimal glands were removed, fixed and then prepared for light and electron microscopic studies. Quantitative studies on lacrimal gland cell micrographs were performed by point counting method. The results were statistically compared between the two groups. **Results:** Light microscopic observation showed many secretory granules in the cytoplasm of the lacrimal gland cells, which were also seen in the lumen of acini. Ultrastructure study of these cells showed the presence of inclusions in their cytoplasm with homogenous and dense structure. In quantitative analysis, the volume fractions (Vv) of mitochondria and nucleus to the cell showed no differences between the two groups but the Vv of euchromatin to the nucleus was different ($P<0.05$). **Conclusion:** The presented results show adverse effects of amiodarone on rabbit lacrimal gland cells. *Iran. Biomed. J. 12 (2): 129-132, 2008*

Keywords: Amiodarone, Lacrimal gland, Ultrastructure, Point counting method

INTRODUCTION

Amiodarone is an iodinated benzofuran derivative anti-arrhythmic drug recommended for the management of ventricular fibrillation and ventricular tachycardia [1, 2]. This drug has also been prescribed for the conversion of atrial fibrillation, maintenance of sinus rhythm and supra ventricular tachycardias [3]. The mechanism of action is to block sodium and calcium channels and β adrenergic receptors in the cells of myocardium and prolongs repolarization [3, 4]. The onset of amiodarone effect is three days to three weeks [1] and it can be accumulated in different tissues [4]. As the use of the drug has become more common, various adverse effects have emerged [5] that one of them is ocular complication. Ocular side effects were first reported in 1967[6] and Bockhardt [7] studied on the effects of amiodarone on retinal pigment epithelium in 1978. However, ocular side effects such as optic neuropathy and keratopathy had been reported since 2001 [8, 9]. Most investigations into the adverse effects of amiodarone on eye have mainly been focused on optic nerve and cornea but not lacrimal gland, which is the accessory apparatus and produces tear. Since the amount of tear secretion affects cornea and visual function and because, to our knowledge, no pathological study on the ultrastructure of lacrimal gland cells following amiodarone administration were reported so far, this research carried out for the study of morphological changes of lacrimal gland cells when amiodarone was applied.

*Corresponding Author; E-mail: femehra@yahoo.com

MATERIALS AND METHODS

Male rabbits (n = 14) with weight of 1.5 kg were purchased from Pasteur Institute of Iran (Tehran, Iran), housed in room controlled at 23 ± 2°c and equally divided into control and experimental groups. Experimental group were intra peritoneally injected with a daily single dose 80 mg/kg amiodarone (EBEWE Pharma, Austria) for two weeks. The control group only received normal saline. At the end of the injection period, the two groups were anesthetized and perfused with Karnovsky's fixative. The lacrimal glands were removed and transferred into Karnovsky's solution for overnight fixation. The tissues were divided into two parts: one for electron microscope and the other for light microscope observations. For electron microscope study (with collaboration of Iran Medical School Electron Microscope Center, Tehran, Iran), fixed tissues were rinsed in 0.1 M phosphate buffer and treated with osmium tetroxide for two hours, then rinsed in phosphate buffer, dehydrated in graded acetone series then infiltrated with TAAB 812 Epon resin and finally embedded and polymerized at 60°c for 48 h. The ultra thin sections were prepared, stained with uranylacetate and lead citrate and then examined with Zeiss Electron Microscope (Germany). The second part of the tissue was processed for light microscope study. The specimen was dehydrated in alcohol series and embedded in paraffin and 5-µm thick sections were cut and stained with Hematoxilin and Eosin.

Quantitative studies. Volume fractions (Vv) of mitochondria, nucleus to the cell and Vv of euchromatin to the nucleus were estimated by means of micrograph image analysis and point counting technique [18, 19].

Statistical analysis. The data obtained by point counting were statistically compared between the control and experimental groups by the student's *t*-test and SPSS software.

RESULTS

The lacrimal glands of control group showed normal cells in appearance. These cells had typical basophilic nucleus and acidophilic cytoplasm. Small normal secretory granules were observed in the cytoplasm and the lumens of acini were clear (Fig. 1). In experimental group an abnormal secretion was observed inside the cells so that the secretory granules led to the formation of vacuoles inside the cells (Fig. 2) and the lumens of acini were full of secretion materials (Fig. 3).The integrity of the nucleus envelope and the morphology of mitochondria were not changed (Fig. 4). Electron microscope studies also showed that the treated animals in comparison to the control group (Fig. 4a), contained inclusions that pressed and localized among the vacuoles with condensed homogen structure (Fig. 5). Quantitative studies of the ultra structure micrographs are summarized in Table 1.
The Vv of mitochondria and nucleus to the cell were statistically not different between the two groups but the difference of Vv for euchromatin to the nucleus between the two groups was significant ($P<0.05$).

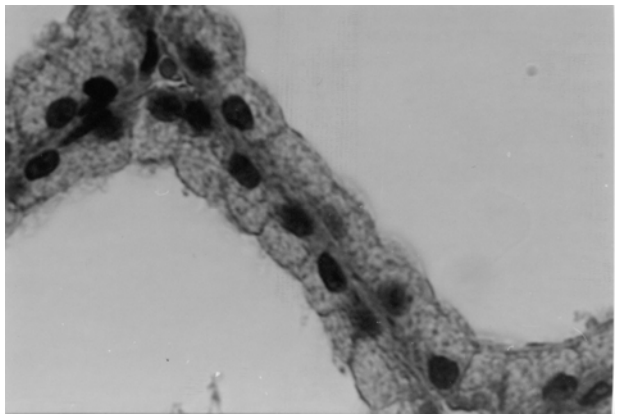

Fig.1. Photomicrograph of lacrimal gland cells in control group. The cells show a normal appearance with acidophilic cytoplasm and basophilic nucleus (×100, H and E staining).

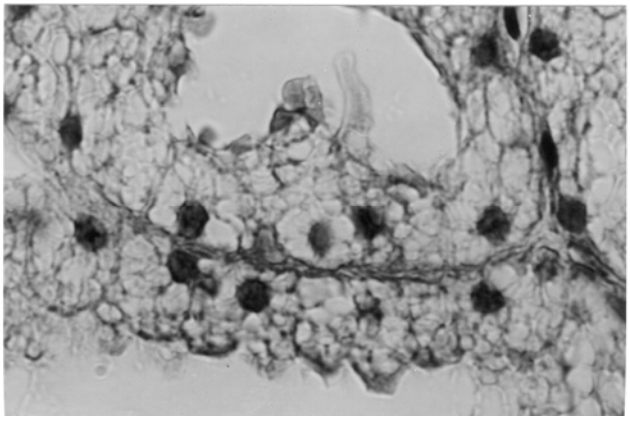

Fig. 2. Photomicrograph of lacrimal gland cells in experimental group .The cells are filled with secretory granules (arrows**)** **(**×100, H and E staining).

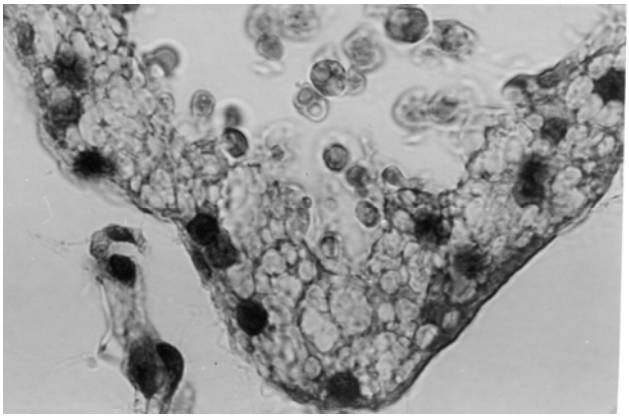

Fig. 3. Photomicrograph shows the secretory particles (arrows) in the lumen of acinous (×100, H and E staining).

DISCUSSION

Studies on amiodarone showed that this drug causes numerous non-cardiac adverse effects [10, 4] so that its toxicity induces pathologic changes in other organ systems [12]. In this study, experimental lacrimal gland cells displayed abnormal features. These cells contained vacuolated cytoplasm and the lumens of acini had secretory materials. The presence of numerous secretory granules in the cells indicates an induction role of amiodarone in gland secretion. Amiodarone is secreted in tear and corneal cells uptake and accumulated the drug in their cytoplasm that cause keratopathy [3]. Electron microscopic observations revealed inclusions among the vacuoles. These results may agree with the investigation represented by Dake *et al.* [17]. They reported the distribution of inclusion bodies in their patients' tissues and suggested that amiodarone induces a systemic metabolic abnormality in lysosomal function that it should be recognized in human tissues. Furthermore, tissue phospholipid content increases in animals receiving these compounds. These studies have suggested that amphiphilic molecules have a preferential attraction for intra lysosomal phospholipids, they strongly bind with these phospholipids and render them indigestible by phospholipases.

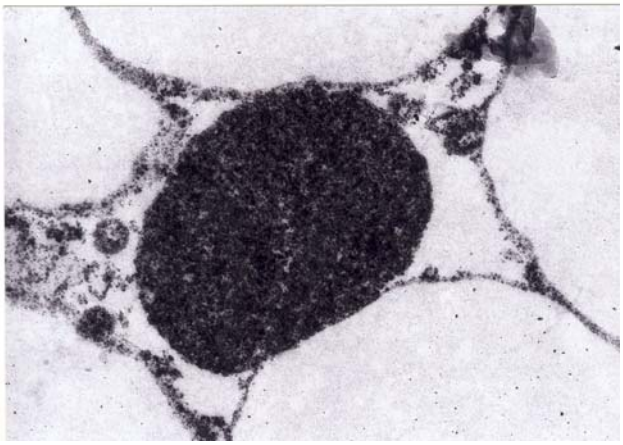

Fig. 5. High magnification of a dense inclusion among the secretory granules (×20,000).

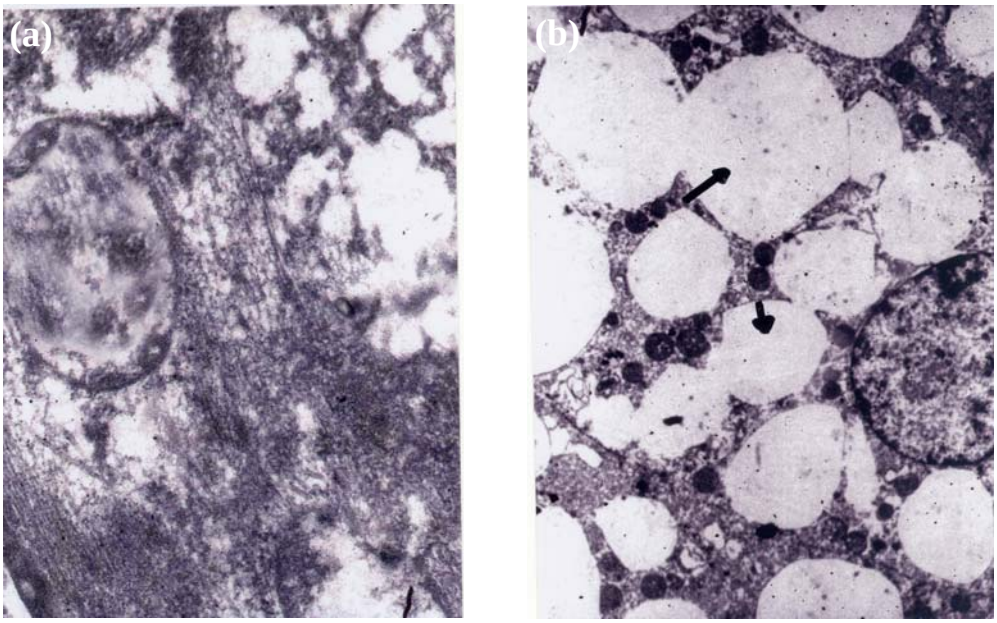

Fig. 4. (a) Electron micrograph of lacrimal gland cell in control group with normal secretory granules in it (×3,000); **(b)** electron micrograph of lacrimal gland cell in experimental group with large secretory granules (arrows) (×3,000).

Table 1. The volume fractions of mitochondria and nucleus to the cell and Vv of euchromatin to the nucleus expressed as means and standard deviations.

Volume fraction	Control group (n = 7)	Experimental group (n = 7)
mitochondria to the cell	0.027 ± 0.010	0.036 ± 0.001
Nucleus to the cell	0.074 ± 0.028	0.095 ± 0.003
Euchromatin to the nucleus	0. 351 ± 0.031	0.375 ± 0.003*

*Significant.

These bound complexes combine to form intra lysosomal inclusion bodies [17, 11]. The lysosomal inclusions formed as a result of impaired phospholipid metabolism could represent either accumulated endogenous cellular material or exogenous material that has been phagocytosed or pinocytosed by the cells [17, 13-15]. There has also been debate in the past as to whether the inclusion bodies in amiodarone treated animals reflect the ongoing cytotoxic process or whether these bodies are directly toxic to the cell in their own right [16].

Quantitative analysis revealed that Vv of euchromatin had increased in experimental group so this finding indicates the increase in cell activity for secretion. We must infer that amiodarone induced morphological changes in lacrimal gland cells as a toxic agent and long time treatment may cause serious ocular side effects. Therefore, appropriate visual screening and monitoring should carry out whenever drug related toxicity is suspected. These results suggest that the consumption of this drug should be restricted and substituted drugs with fewer side effects should be used.

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