Atrophy of Rat Exorbital Lacrimal Glands Induced by Atropine

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Abstract: To assess the morphological changes of the lacrimal gland after cholinergic suppression, we performed light- and transmission-electron microscopic examinations on the exorbital lacrimal glands in rats after intragastric intubation of atropine, a nonselective antimuscarinic compound with a high affinity for M3 muscarinic subtype receptors. In addition, areas of acinar cells were measured by an image processor. The weights of the lacrimal gland were decreased at hours 36 and 48 after atropine single administration at 250 mg/kg. Areas of acinar cells, measured by an image processor, were also decreased. Electron-microscopic examination demonstrated decreased number of secretory granules in the apical cytoplasm of acinar cells in all rats on days 7 and 28 of repeated atropine treatment. No changes were evident in other cytoplasmic organelles. Four- and even thirteen-week repeated administration studies revealed no progression in lacrimal acinar cell atrophy. The present findings demonstrate that an antimuscarinic compound that has high affinity for M3 muscarinic subtype receptors induces lacrimal gland acinar cell atrophy caused by a decrease in secretory granules, and that the repeated administration is not associated with any progression or any other changes. (J Toxicol Pathol 2000; 13: 7-11)

Key words: lacrimal gland, atrophy, atropine, cholinergic antagonist, rat

Introduction

The tubuloalveolar exocrine acinar cells of the exorbital lacrimal gland are polyhedral or pyramidal with round to oval nuclei, and secrete proteins, electrolytes, and water1,2. Innervation is by both parasympathetic and sympathetic nerves, with the former predominating2. It is well known that muscarinic cholinergic receptors control the production of lacrimal protein and fluids, and muscarinic antagonists inhibit their secretion4-6. Acetylcholine, a major neurotransmitter of parasympathetic nerves, stimulates the muscarinic cholinergic pathway8. Recently, many pharmacological and genetic studies have indicated that M3 muscarinic subtype receptors predominantly control lacrimal gland secretion7,9. However, reports on the morphological changes of the lacrimal gland after single or repeated treatment with muscarinic antagonists are limited. We, therefore, performed light- and transmission electron-microscopic examinations on the lacrimal glands in rats after single and repeated exposure to atropine, a classical nonselective antimuscarinic compound with a high affinity for M3 muscarinic subtype receptors. Morphometric examination on the lacrimal glands in rats after single exposure to atropine was also performed.

Materials and Methods

Animals

A total of 166 male Slc: SD rats (purchased from Japan SLCo., Ltd., Hamamatsu), 6 weeks old at the commencement of treatment, were used. The animals were individually housed in stainless steel cages and had free access to pellet feed (CRF-1, Oriental Yeast Co., Ltd., Tokyo) and tap water. The animal room was controlled to maintain a temperature of 23±2°C and a humidity of 55±10% with 10-35 times/hour ventilation, and a lighting period 7:00-19:00.

Test compound

Atropine sulfate, obtained from Tokyo Chemical Industry Co. Ltd., Tokyo, was of technical grade with a purity of 97.1%. It was dissolved in distilled water to give a 10% w/v solution.

Experimental Protocols

Experiment 1

A single dose of the test article at 250 mg/kg was given orally using a gastric tube. Five rats each were weighed and euthanized by exsanguination under ether anesthesia at 2, 6, 12, 18, 24, 36, and 48 hours after dosing, along with five non-treated rats each at the 0, 6, 12, and 18 hour time points as controls. Bilateral exorbital lacrimal glands were removed, weighed, and fixed in 10% buffered formalin. They were examined under a light microscope after a routine process of embedding in paraffin and staining with hematoxylin-eosin. Two areas were chosen randomly each from two sections (each one section from right and left exorbital lacrimal glands) and the areas of acinar cells were measured using a color video image processor (SP500, Olympus Optical Co. Ltd., Tokyo). The numbers of the lacrimal acinar cells in...
Table 1. Exorbital Lacrimial Gland Weights in Rats Treated Orally with Atropine for 1, 2, 4, or 13 Weeks (Experiment 3)

<table>
<thead>
<tr>
<th>Administration period (week)</th>
<th>Group</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Bilateral exorbital lacrimial gland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Absolute weight (g)</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>12</td>
<td>215±9**</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>4</td>
<td>182±12**</td>
<td>0.16±0.02**</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>12</td>
<td>254±10</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>12</td>
<td>222±16**</td>
<td>0.16±0.02**</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>12</td>
<td>319±28</td>
<td>0.25±0.02</td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>11</td>
<td>277±19**</td>
<td>0.16±0.02**</td>
</tr>
<tr>
<td>13</td>
<td>Control</td>
<td>14</td>
<td>458±41</td>
<td>0.27±0.04</td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>13</td>
<td>380±45**</td>
<td>0.18±0.03**</td>
</tr>
</tbody>
</table>

** Mean±SD
Significantly different from the control group: **p<0.01

the measured areas were counted to determine the area of an individual acinar cell (μm²/cell). The measured areas of each rat included at least more than 500 cells in all.

Experiment 2
Atropine sulfate at 250 mg/kg was repeatedly administered using a gastric tube to rats once a day until euthanasia. Three rats each were euthanized after 7 and 28-day administrations. Three non-treated rats were also sacrificed after 14 days as a control group. A small strip dissected from the fresh lacrimal gland was fixed in 3% glutaldehyde, post-fixed with 1% osmic acid, and embedded in Epon for electron microscopic examination. Ultra-thin sections were prepared, stained by uranyl acetate and lead citrate, and observed under a transmission electron microscope (Model JEM-100CX, JEOL, Tokyo).

Experiment 3
For the first three days, atropine sulfate at 500 mg/kg was administered daily using a gastric tube for rats. Because this resulted in death of 8 of 51 rats, the dose level was reduced to 250 mg/kg for the remaining treatment period. Four rats were euthanized after one week, and 11–13 rats each after 2, 4, and 13 weeks of administration. Control rats (12–14 animals) given distilled water daily were also euthanized at the same time points (Table 1). The bilateral exorbital lacrimial glands were removed, weighed, and fixed in 10% buffered formalin, embedded in paraffin and stained with hematoxylin–eosin for histological examination.

Statistic analysis
Variance in all measured values was compared between atropine–treated and control groups by the F test. Homogeneous data were analyzed by the Student’s t-test, and non-homogenous data by the Aspin-Welch’s t-test. The SAS statistical program (SAS Institute, Inc., Cary, NC, USA) was employed for this purpose.

Results

Experiment 1
Data for atropine–treated rats at the 2 hour point were compared with the control values at zero hour, the 6, 12, and 18 hour points with concurrent controls and the 24, 36, and 48 points with the control values at 18 hours.

Body weights of atropine–treated rats exhibited similar values to control rats until hour 24, but were slightly decreased at hours 36 and 48. The lacrimal gland weights were slightly increased in rats until 18 hours after administration, but the values were not statistically significant. They were decreased slightly in rats at hour 24, and decreased with statistical significance at hours 36 and 48 after atropine administration (Fig. 1). The decreases were especially prominent in weights in relation to body weights at the 48 hour point (Atropine: 79±8 mg/100 g body weight, Control: 18hr: 106±11 mg/100 g body weight). Microscopically, atrophy of the acinar cells, characterized by a decrease in clear cytoplasm in their apical region, was evident in those rats (Figs. 2a, b). Areas of acinar cells in the treated rats were similar to the control value until 24 hours, but they were decreased significantly at hours 36 and 48 (Fig. 3). There were no histological changes in other cells surrounding acini.
Experiment 2

Acinar cells in control rats had many secretory granules at the apex and rough endoplasmic reticulum in the basal cytoplasm (Fig. 4a). Decreases in number of secretory granules in the acinar cells were apparent in all rats after 7 and 28 days of atropine treatment (Fig. 4b). There were no differences in the degree of the decrease between the two time points. No changes in other cytoplasmic organelles of acinar cells were apparent in the atropine-treated rats as compared with the controls. There were no ultrastructural alterations in myoepithelial cells surrounding acini.

Experiment 3

On the second or third day of atropine treatment at 500 mg/kg, 8 of 51 rats died after jumping, convulsion, tremor, deep respiration, prone position, or hypothermia. The cause of death was surmised to be central nerve toxicity of large amount of the atropine-treatment. Atrophy of the acinar cells of exorbital lacrimal gland was evident in 5 of 8 dead rats.

Body weights of atropine-treated rats were decreased during the administration period as compared with the control values (Table 1). Absolute and relative exorbital lacrimal gland weights were also significantly decreased in rats as early as week 2 and remained low after 13 weeks of atropine administration (Table 1). However, the weight ratios to the control values at week 13 were comparable to those at weeks 2 and 4. Microscopically, acinar cell atrophy was apparent in atropine-treated rats (Fig. 2c) with no marked difference among examination points. Thus, the prolonged treatment had little effect on the degree of acinar cell atrophy. No other changes were found in the lacrimal gland of atropine-treated rats.

Discussion

The present study of the lacrimal glands in rats after a single exposure to atropine, which has high affinity for M3 muscarinic subtype receptors, demonstrated atrophy of acinar cells. This finding was mainly due to a decrease in secretory granules in the apical part of the cytoplasm at hours 36 and 48. The secretory granules contain the protein synthesized in endoplasmic reticulum and modified in the Golgi apparatus. Muscarinic agonists activate a calcium signaling and protein kinase C-dependent pathway so that protein synthesis in the endoplasmic reticulum was stimulated. Our results are in line with inhibition of signal transduction induced by atropine through its binding to M3 muscarinic subtype receptors.

Hollinworth et al. reported results of Schirmer tear test of the dogs in case of topical administration of atropine. Decreased tear production was most marked 2 hours after...
Fig. 4. Electron micrographs of acinar cells of exorbital lacrimal glands. Normal appearance in a control rat in experiment 2 (a). Evident decrease of number of secretory granules in a rat after 28 days of atropine-repeated treatment in experiment 2 (b). Bar = 2 μm
atropine instillation, with return to normal baseline values within 6 hours. However, our study demonstrated no significant differences in the morphology of the lacrimal gland between atropine-treated and control rats until 18 hours after a single atropine oral administration, suggesting a much slower action via the systemic route. This then persisted longer, as evidenced by the statistically significant decrease in lacrimal gland weight relative to body weight in rats at 48 hours.

Similar results were obtained with repeated administration of atropine. Electron microscopic examination demonstrated decreases in the number of secretory granules in the acinar cells. Interestingly, there were no differences in the degree of the atrophic effects between 7 and 28 days of atropine treatment. Although Lemullos reported15 that the myoepithelial cells have more receptors than the acinar cells, our examinations did not demonstrate histological changes in the myoepithelium.

Dethloff et al.16 reported that four-week treatment with a muscarinic agonist induced hypertrophy of lacrimal and Harderian gland in rats. In contrast to the muscarinic agonist case, we demonstrated in Experiment 3 that even 13-week-repeated application of atropine, muscarinic antagonist, did not affect the degree of atrophy or the extent of the histopathological changes in the exobital lacrimal glands.

In conclusion, this study demonstrated that atrophy of the lacrimal acinar cells with decreases in the number of secretory granules occurs in rats within 36 hours of a single atropine treatment. A similar change was also found in rats repeatedly treated with atropine. However, the prolonged treatment for up to 13 weeks did not cause progressive atrophy of acinar cells and no other changes were induced in the lacrimal glands.

References