Radioprotective effect of montelukast sodium in rat lacrimal glands after radioiodine treatment

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ABSTRACT

Purpose: The aim of this study was to evaluate the morphological changes of rat lacrimal glands at the third month following radioiodine (RAI) treatment and the radioprotective effect of montelukast (ML) sodium against RAI-related lacrimal gland damage.

Methods: Fifty female Wistar Albino rats were divided into three groups. The control group (n = 10) consisted of rats with no intervention. RAI group (n = 20) consisted of rats treated with oral 131I (111 MBq). The ML group (n = 20) consisted of rats treated with intraperitoneal 10 mg/kg/day ML sodium, starting three days before and continuing for one week after RAI administration. Intraorbital (IG), extrabulbar (EG) and Harderian glands (HG) were removed bilaterally after three months.

Results: The existence of acinar atrophy, acinar fibrosis, abnormal cell lines, peripheral basophilic, cell size variation and decrease in amount of cytoplasm was significantly more common in the RAI-rat treated group than in the ML group, in each of the glands. The ML-treated group had less-frequent cell shape variation in EG (P = 0.001) and HG (P = 0.027), cell size variation in IG (P < 0.001) and HG (P = 0.01), ductal pathology in EG (P < 0.001) and HG (P < 0.001) and lipofuscin accumulation in EG (P = 0.001) and in HG (P = 0.01) than the RAI-treated group.

Conclusions: RAI treatment seems to cause morphological damage to rat lacrimal glands, and ML sodium effectively protects against damage to lacrimal glands.

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Efecto radioprotector de montelukast sódico en glándulas lagrimales de las ratas después del tratamiento con yodo radioactivo

RESUMEN

Objetivo: El objetivo de este estudio fue evaluar los cambios morfológicos de las glándulas lagrimales de las ratas en el tercer mes después del tratamiento con iodo radioactivo (RAI) y el efecto radioprotector de montelukast (ML) sódico contra los daños causados por el RAI en la glándula lagrimal.

Métodos: Cincuenta ratas hembras de raza Wistar fueron divididas en 3 grupos. El grupo de control (n = 10) estaba formado por ratas no intervenidas. El grupo de RAI (n = 20) estaba formado por ratas tratadas con 131I orales (111 MBq). El grupo ML estaba formado por ratas tratadas con 10 mg/kg/d de ML sódico intraperitoneal comenzando 3 días antes y continuando durante una semana después de la administración oral del RAI. Las glándulas intraorbitarias (IG), las extrabulbar (EG) y las de Harder (HG) se eliminaron bilateralmente en 3 meses.

Resultados: La existencia de atrofia acinar, fibrosis acinar, líneas celulares anormales, basofilia periférica, la variación de tamaño de las células y la disminución de la cantidad de citoplasma eran significativamente más común en todas las glándulas separadas en el grupo de ratas tratadas con RAI que en el grupo de ratas tratadas con ML. El grupo tratado con ML presentaba menor variación frecuente de la forma celular en las EG (P = 0.001) y las HG (P = 0.027), variación de tamaño de las células en las IG (P = 0.001) y las HG (P = 0.01), enfermedad ductal en las EG (P < 0.001) y las HG (P < 0.001) y menor acumulación de lipofusina en las EG (P = 0.001) y en las HG (P = 0.01) que el grupo tratado con RAI.

Conclusions: El tratamiento con RAI parece causar daño morfológico en las glándulas lagrimales de las ratas y el ML sódico protege eficazmente a las glándulas lagrimales de ese daño.

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Introduction

Radioiodine (RAI, $^{131}\text{I}$) has been used for the treatment of hyperthyroidism and well-differentiated thyroid carcinomas.\textsuperscript{1,3} Some short-, intermediate- and long-term complications of RAI treatment have been identified.\textsuperscript{2,4} The short-term complications are xerostomia, nausea, gastralgia, pain in the thyroid bed, tenderness over the parotid gland and submandibular glands, change in taste and vomiting.\textsuperscript{1} The intermediate complications are sialoadenitis, loss of taste or smell and transient alopecia.\textsuperscript{2} The long-term complications are xerostomia, xerophthalmia, chronic or recurrent conjunctivitis and dacyrostenosis\textsuperscript{2,3,5,6}

Xerophthalmia has been reported to occur in 7.6–92% of the patients after RAI treatment.\textsuperscript{5,6} Although the concentration of $^{131}\text{I}$ in lacrimal glands has not been calculated, it has been reported that $^{131}\text{I}$ is secreted into tears and causes lacrimal gland damage\textsuperscript{3,7–9} in 1988 and 1991, Stephens et al. performed the first experimental study on rhesus monkeys to determine the morphological causes of xerophthalmia after radiation therapy, and they determined cell death within 24 h of radiation.\textsuperscript{7,10} Following these experimental studies, the first case with lacrimal dysfunction after RAI therapy was reported in 1993.\textsuperscript{3} Bakheet et al. later reported $^{131}\text{I}$ accumulation in RAI in the lacrimal sac of a patient with dacriocystitis.\textsuperscript{9} Consequently, the same team showed and calculated $^{131}\text{I}$ secretion in tears for that patient.\textsuperscript{3}

The precise mechanism of lacrimal gland damage is still unknown, although Na+/I− symporter (NIS) function is presumed to be involved. It has been demonstrated through immunohistochemical analyses that NIS protein is expressed in lacrimal glands. This suggests that $^{131}\text{I}$ is taken into lacrimal gland cells via NIS, and this may be the main cause of lacrimal gland dysfunction following RAI therapy.\textsuperscript{11} Moreover, ionizing radiation transmitted from adjacent organs can lead to additional damage. Ionizing radiation both from $^{131}\text{I}$ in lacrimal cells and from adjacent organs leads to reactions that produce free oxygen radicals which are the main cause of unwanted RAI-induced oxidative damage.\textsuperscript{12,13} Free oxygen radicals can interact and destroy cell membranes and organelles and eventually, cells.\textsuperscript{12,13} Depending on this oxidative mechanism, most of the radioprotective agents studied so far have been anti-oxidants. The only approved drug by the Food and Drug Administration (amifostine) for radiation-induced xerostomia is also an antioxidant.\textsuperscript{14}

The rationale of this study was the knowledge that RAI causes tissue damage by oxidative mechanisms and the fact that montelukast (ML) has antioxidative effects. In addition, there are no agents that have been studied as potential radioprotectors for radioimmune-related lacrimal gland damage. The aim of this study was to investigate the RAI-induced lacrimal gland changes and the radioprotective effect of ML in rat lacrimal glands.

Material and methods

This experimental study was approved by the Local Ethics Committee of Animal Experiments (Ankara Training and Research Hospital, Ankara, Turkey) and conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. This study was conducted in the Hüsnü Sakal Experimental and Clinical Practice Center, Ankara, Turkey.

Animals

The animals were purchased from Saki Yenilli Experimental Animal Production Laboratory (Ankara, Turkey). The study was carried out on 50 female Wistar Albino rats (weight range: 225–275 g, age range: 4–6 months). The rats were acclimated for at least 1 week before the study. The animals were housed under standard laboratory conditions with constant temperature (21 ± 2°C) and a relative humidity of 65–70% with 12-hour light and dark cycles. The rats were housed in polypropylene cages using disposable absorbent cloths under sterile paddy husks to avoid contamination from radioactive urine. The animals were fed with standard chow and water ad libitum.

Experimental design

The animals were assigned to one of the three groups in a random order. The first group was the untreated control group (n = 10). The rats in the second group (RAI group, n = 20) were treated with oral $^{131}\text{I}$ (111 MBq). The rats in the third group (ML group, n = 20) were treated with an intraperitoneal radioprotective agent (10 mg/kg/day, ML sodium; Merck Sharp and Dohme, Istanbul, Turkey) for three days, then oral $^{131}\text{I}$ (111 MBq) was applied and intraperitoneal ML sodium 10 mg/kg/day was continued for one week following the $^{131}\text{I}$ treatment. On the third month of $^{131}\text{I}$ administration, the animals were anesthetized with 50 mg/kg, i.p. propofol (Abbott Laboratuvari Anonim Sirketi, Istanbul, Turkey) and decapitated afterwards.

Intraorbital (IG), extraorbital (EG) and Harderian glands (HG) were removed bilaterally. The thyroid glands of all the rats treated with RAI were atrophied, and no pathologically detectable normal thyroid tissue persisted.

Pathology

The lacrimal glands were fixed in 10% neutral buffered formalin (pH 7.2–7.4) for light microscopy and 4-μm-thick paraffin sections were stained with hematoxylin and eosin. The specimens were evaluated using light microscopy (Olympus BX-50, Tokyo, Japan) at 40–400-fold magnification in a masked fashion. The first three sections and every 10th section thereafter were stained. All of the stained sections, approximately 15 slides per specimen for the right and left lacrimal glands, were studied (Fig. 1).

The histopathological changes were evaluated according to previously published grading systems: Acinar atrophy (Grade 0: no atrophy, Grade 1: less than 50% acini atrophic, Grade 2: more than 50% acini atrophic, Grade 3: all acini atrophic, Grade 4: uncertain),\textsuperscript{15} acinar fibrosis (Grade 0: absent, Grade 1: only within one lobule, Grade 2: in less than 50% of the lobules, Grade 3: in more than 50% of the lobules),\textsuperscript{16} ductal pathology (Grade 0: all ducts normal, Fig. 1. Normal lacrimal gland morphology in the control group (hematoxylin and eosin 20× magnification; bar: 50 μm).
Table 1
Distribution of histological inflammatory parameters in control, montelukast protected and non-protected animal groups according to the rat lacrimal gland types and their statistical significance levels.

<table>
<thead>
<tr>
<th>Inflammatory parameters</th>
<th>Control group (n = 10)</th>
<th>Radioiodine group (n = 20)</th>
<th>Montelukast group (n = 20)</th>
<th>Gland type; P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IG  n (%)</td>
<td>EG  n (%)</td>
<td>HG  n (%)</td>
<td>IG  n (%)</td>
</tr>
<tr>
<td>Lobular pattern</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>9 (90)</td>
<td>9 (90)</td>
<td>7 (70)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td>3 (30)</td>
<td>16 (18)</td>
</tr>
<tr>
<td>Acinar atrophy</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>20 (100)</td>
</tr>
<tr>
<td>No</td>
<td>9 (90)</td>
<td>10 (100)</td>
<td>9 (90)</td>
<td>-</td>
</tr>
<tr>
<td>Ductal pathology</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>11 (55)</td>
</tr>
<tr>
<td>No</td>
<td>100 (10)</td>
<td>100 (10)</td>
<td>100 (10)</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Lipofuscin</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>13 (65)</td>
</tr>
<tr>
<td>No</td>
<td>9 (90)</td>
<td>100 (10)</td>
<td>100 (10)</td>
<td>7 (35)</td>
</tr>
</tbody>
</table>
| Grade 1: less than 50% ducts abnormal, Grade 2: more than 50% ducts abnormal, Grade 3: all ducts abnormal, Grade 4: uncertain and lipofuscin accumulation (Grade 0: not present, Grade 1: within only few lobules, Grade 2: in less than 50% of the lobules, Grade 3: in more than 50% of the lobules).16

Data analysis

Data analysis was performed using Statistical Package for Social Sciences for Windows software (SPSS version 15.0, SPSS Inc., Chicago, USA). All of the variables were categorical. The descriptive statistics were expressed in frequency (%). The RAI group and ML group were compared with Chi square test or Fisher’s exact test.

Results

The existence of acinar atrophy (P < 0.001, for all the glands) (Table 1), acinar fibrosis (P = 0.022, P = 0.002, P < 0.001 for IG, EG, HG) (Table 1, Fig. 2), abnormal cell outlines (P < 0.001 for all the glands) (Table 2), scant cytoplasm (P = 0.027, P = 0.004, P = 0.004 for IG, EG, HG) (Table 2), cell size variation (P < 0.001, P = 0.038, P = 0.01, for IG, EG, HG) (Table 2) and peripheral basophilia (P = 0.001, P = 0.008, P = 0.009 for IG, EG, HG) (Table 3) was less frequently observed in the ML Group than in the RAI Group.

The cell shape variation in EG (P = 0.001) and HG (P = 0.027), cell size variation in IG (P < 0.001) and HG (P = 0.01), ductal pathology in EG (P < 0.001) (Fig. 2) and HG (P < 0.001) and lipofuscin accumulation in EG (P = 0.001) (Fig. 2) and HG (P = 0.01) (Tables 1–3) were less frequently observed in the ML Group than in the RAI Group.

The RAI Group and the ML group had similar cell shape variation in IG, ductal pathology in IG, lymphocytic infiltration in IG and in HG, polymorphonuclear infiltration in all the glands and lipofuscin accumulation in IG (Tables 1–3).

Comparison of the nucleus characteristics of rat lacrimal glands between montelukast protected and non-protected animals is presented in Table 4 and Fig. 3.

Discussion

The current study is the first to describe RAI-induced changes in rat lacrimal glands at the third month after oral 131I (111 MBq) administration and the effect of ML in preventing these changes. Lacrimal gland dysfunction is one of the late complications of 131I therapy. This complication, which is reported to occur in 7.6–92% of patients, can be permanent.16,17 Bakheet et al. demonstrated in a single case that about 0.01% of the ingested 131I can be secreted through tears within the first 4 h.8 Zetting and colleagues measured the radioactivity of contact lenses in a single patient and demonstrated radioactivity on the first day, which diminished to negligible amounts over the following days.18 The literature contains four clinical studies evaluating the effect of RAI on lacrimal gland function.2,5,6,17 Alexander et al. reported that 131I causes ocular side effects, having determined this by evaluating a questionnaire including a question asking patients whether they suffer often or continuously from red eyes, conjunctivitis or increased lacrimation; however, in this study discrimination between aqueous dry eye and other complications was not made.7 A prospective cohort (n = 79 patients) study was conducted to determine the prevalence of lacrimal gland dysfunction after RAI treatment. In that study, objective xerophthalmia was diagnosed when two of three tests (Schirmer’s test, rose bengal staining and tear break-up time) were abnormal.9 According to the results of that cohort, subjective xerophthalmia occurred in 25.3%
and 13.9% of patients during the first and third year, respectively and objective xerophthalmia occurred in 17.7% and 7.6% of patients during the first and third year, respectively. In that cohort, xerophthalmia developed in a significant percentage of patients, who mostly recovered thereafter while some appeared later. Zetting et al. performed a cross-sectional study (n = 88 patients) and commented that lacrimal gland function may be permanently impaired after high-dose RAI treatment. They found that 92% of patients had at least one abnormal tear function test (Schirmer’s test, tear break-up time, lipid layer interference pattern) after 64 ± 71 months (min–max: 3–317 months), and abnormal tear film function was found to be significantly abnormal (P < 0.001) as compared to age-sex-matched controls. Zetting et al. reported a higher percentage of abnormal Schirmer’s test results (<5 mm/5 min) (40% vs. 18%), tear break-up time (71% vs. 13%) and abnormal lipid layer (49% vs. 10%) than Solans et al. after RAI treatment. The difference

![Fig. 2](http://www.elsevier.es)

### Table 2

<table>
<thead>
<tr>
<th>Morphologic parameters</th>
<th>Control group (n = 10)</th>
<th>Radioiodine group (n = 20)</th>
<th>Montelukast group (n = 20)</th>
<th>Gland type; P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IG n (%)</td>
<td>EG n (%)</td>
<td>HG n (%)</td>
<td>IG n (%)</td>
</tr>
<tr>
<td><strong>Cell outlines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>10(100)</td>
<td>8(80)</td>
<td>6(60)</td>
<td>4(20)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>–</td>
<td>2(20)</td>
<td>4(40)</td>
<td>16(80)</td>
</tr>
<tr>
<td><strong>Cell shape variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1(10)</td>
<td>–</td>
<td>1(10)</td>
<td>7(35)</td>
</tr>
<tr>
<td>No</td>
<td>9(90)</td>
<td>10(100)</td>
<td>9(90)</td>
<td>13(65)</td>
</tr>
<tr>
<td><strong>Cell size variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>–</td>
<td>1(10)</td>
<td>–</td>
<td>18(90)</td>
</tr>
<tr>
<td>No</td>
<td>100(10)</td>
<td>9(10)</td>
<td>100(10)</td>
<td>2(10)</td>
</tr>
<tr>
<td><strong>Amount of cytoplasm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>9(90)</td>
<td>8(80)</td>
<td>7(70)</td>
<td>8(40)</td>
</tr>
<tr>
<td>Scant</td>
<td>1(10)</td>
<td>2(20)</td>
<td>3(30)</td>
<td>12(60)</td>
</tr>
</tbody>
</table>

IO: intraorbital lacrimal gland; EO: extraorbital lacrimal gland; HG: Harderian gland; NS: not significant.

*P* value: radioiodine group versus montelukast group.
Table 3
Distribution of cellular parameters in control, montelukast protected and non-protected groups according to the rat lacrimal gland types and their statistical significance levels.

<table>
<thead>
<tr>
<th>Cellular parameters</th>
<th>Control group (n = 10)</th>
<th>Radioiodine group (n = 20)</th>
<th>Montelukast group (n = 20)</th>
<th>Gland type; P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IG  n (%)  EG  n (%)  HG  n (%)</td>
<td>IG  n (%)  EG  n (%)  HG  n (%)</td>
<td>IG  n (%)  EG  n (%)  HG  n (%)</td>
<td></td>
</tr>
<tr>
<td>Peripheral basophilia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8(80)  8(80)  8(80)</td>
<td>6(30)  9(45)  9(45)</td>
<td>17(85)  17(85)  17(85)</td>
<td>IG: &lt;0.001</td>
</tr>
<tr>
<td>Abnormal</td>
<td>2(20)  2(20)  2(20)</td>
<td>14(70) 11(55) 11(55)</td>
<td>3(15)  3(15)  3(15)</td>
<td>EG: &lt;0.05</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3(30)  1(10)  2(20)</td>
<td>10(50) 9(45) 4(20)</td>
<td>3(15)  2(10)  3(15)</td>
<td>HG: &lt;0.05</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7(70)  9(90)  8(80)</td>
<td>10(50) 11(55) 16(80)</td>
<td>17(85) 18(90) 17(85)</td>
<td></td>
</tr>
<tr>
<td>Polymorphonuclear cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>5(25)  1(5)  2(10)</td>
<td>1(5)  1(5)  –</td>
<td>IG: NS</td>
</tr>
<tr>
<td>No</td>
<td>10(100) 10(100) 10(100)</td>
<td>15(75) 19(95) 18(90)</td>
<td>19(95) 19(95) 20(100)</td>
<td>EG: NS</td>
</tr>
<tr>
<td>Plasma cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2(20)  –  1(10)</td>
<td>9(45)  8(40) 10(50)</td>
<td>1(5)  3(15)  3(15)</td>
<td>IG: &lt;0.05</td>
</tr>
<tr>
<td>No</td>
<td>8(80)  10(100) 9(90)</td>
<td>11(55) 12(60) 10(50)</td>
<td>19(95) 17(85) 17(85)</td>
<td>EG: NS</td>
</tr>
</tbody>
</table>


* P value: radioiodine group versus montelukast group.

Table 4
Distribution of histological findings according to the nucleus characteristics of rat lacrimal glands in the control group, radioiodine group and montelukast group.

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Control group (n = 10)</th>
<th>Radioiodine group (n = 20)</th>
<th>Montelukast group (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IG  n (%)  EG  n (%)  HG  n (%)</td>
<td>IG  n (%)  EG  n (%)  HG  n (%)</td>
<td>IG  n (%)  EG  n (%)  HG  n (%)</td>
</tr>
<tr>
<td>Small with smooth rounded shapes</td>
<td>9(90)  9(90)  9(90)</td>
<td>5(25)  6(30)  8(40)</td>
<td>13(65) 16(80) 17(85)</td>
</tr>
<tr>
<td>Large single (irregular nodular shapes)</td>
<td>1(10)  1(10)  1(10)</td>
<td>10(50) 12(60) 6(30)</td>
<td>6(30)  4(20)  3(15)</td>
</tr>
<tr>
<td>Large double (irregular nodular shapes)</td>
<td>0(10)  0(10)  0(10)</td>
<td>5(25)  2(10)  6(30)</td>
<td>1(5)  0(10)  0(10)</td>
</tr>
</tbody>
</table>


in the results of these two studies may be related to their different study designs, follow-up times and definitions of parameters. Fard-Esfahani et al. found significantly reduced Schirmer scores in RA-exposed patients than in non-exposed patients; however, there was no significant relationship between Schirmer scores and 131I cumulative doses.17

While the precise mechanism of RAI-induced lacrimal gland damage is still unknown, accumulation of 131I in lacrimal gland cells via NIS is presumed to be the trigger of the damage.11 In addition, radiation from adjacent organs containing 131I may contribute to the damage. Ionizing radiation causes tissue destruction mainly by oxidative damage. Free radicals (e.g. superoxide radical, hydrogen peroxide and hydroxyl radical), which are produced by ionizing radiation, interact with intracellular structures and cell membranes and consequently cause cell destruction.12–14,19,20 Apoptosis, cell necrosis and inflammation have been reported as
accompanying events. In terms of RAI-related injury, necrosis is cell death produced by toxic levels of RAI. This type of cell death leads to inflammation that causes additional tissue damage, and the process can be painful for the patient. Apoptosis is programmed cell suicide induced by radiation. Therefore, necrosis is detected immediately after RAI application, and apoptosis is detected at the second day in cell culture.21

Necrosis that occurs immediately after irradiation has been reported in an in vitro study on well-differentiated papillary thyroid carcinoma cell-line incubated at higher 131I concentrations; however, because of the in vitro nature of the study, the associated inflammation could not be evaluated.21 Stephens et al. demonstrated acute neutrophilic inflammation 24 h after external radiation in lacrimal glands of rhesus monkeys.7,10 In contrast, Takagi et al. concluded that massive cell loss does not occur and a decrease in saliva secretion is the result of diminished exocytosis and water secretion after 2–14 days of X-irradiation in rat submandibular gland cells.22 Although pain in the parotid region is frequent, no study has reported pain in the lacrimal gland region after external radiation or RAI treatment; this may reflect absence or minor necrosis, minor sub-sequent inflammation or dose dependence.2,4,23 As the serious acinar cells are mature non-dividing cells with a life span of more than one month, their death at hour 24 can be explained by interphase death, which is dose dependent, rather than reproductive death.24 Damage to stem cells that are involved in the repair of lacrimal gland cells can lead to insufficient replacement of acinar cells that have died due to expired normal life span, radiation-related apoptosis or radiation-induced immediate toxicity and may contribute to long-term lacrimal dysfunction.2,4,24

Apoptosis that possibly arises due to oxidative stress has been demonstrated in monkey lacrimal and salivary glands after external radiation,7 in rat thyroid tissue after 131I treatment25 and in in vitro papillary thyroid carcinoma cells incubated with 131I.21 We did not find possible necrotic, inflammatory and apoptotic reactions in our specimens probably because of the late sacrifice (at the third month) which is too late to find such reactions.

Although few case reports and clinical studies agree that RAI leads to lacrimal gland dysfunction, the literature lacks consistent data on the early and late morphological changes of human and animal lacrimal glands. Moreover, histological description is possible only in animal studies. The only data on morphological alterations are from externally irradiated monkey lacrimal glands after 24 and 48 h of irradiation that describes apoptosis, necrosis and neutrophilic inflammation.7,10 The single report concerning the effect of 131I on human cells is an in vitro study on well-differentiated papillary thyroid carcinoma cell-line, which demonstrated apoptosis at lower concentrations that occurs at the second day and necrosis at higher concentrations that occurs immediately after irradiation.21 Our study describes the RAI-induced changes in rat lacrimal glands during the third month. RAI treatment leads to a decrease in peripheral basophilia and scanty cytoplasm, acinar atrophy and fibrosis, ductal abnormality and lipofuscin accumulation.

As the radioiodine-related damage has been related mainly to oxidative stress, most of the radioprotective agents studied so far have been anti-oxidants.14 The only available radioprotector in clinical use is amifostine that is also an anti-oxidant.14 ML, a leukotriene D4 antagonist, has been studied widely as a protective anti-oxidative and anti-inflammatory agent in various conditions one of which is ischemia reperfusion injury.26

According to our results, histopathological examinations revealed that ML protects rat lacrimal glands against radioiodine-related lacrimal gland damage. Cell outlines, the amount of cytoplasm and peripheral basophilia were better preserved in the ML group, and the difference between the groups was statistically significant. The presence of acinar atrophy, ductal atrophy, acinar fibrosis and lipofuscin accumulation was significantly less frequent in the lacrimal glands of ML-protected rats.

Although data on the leukotriene concentrations in lacrimal or any other radioiodine-treated tissue are not present in the literature, it is possible that ML, as a leukotriene D4 (a proinflammatory cytokine) antagonist, has an additional protective action by inhibiting possible early neutrophilic inflammation. ML probably prevents inflammation by blocking the cysteinyl leukotriene receptor type 1, which is found in neutrophils.27 ML has been shown to down-regulate the human monocyte chemotaxins and to inhibit production of tumor necrosis factor-alpha (TNF-α) and monocyte chemotactic protein-1.28 In another study, it has been reported that ML depressed the TNF-α response in sepsis-induced ileal and hepatic injury in rats.29

The plasma cells were significantly lower in the ML group than in the RAI group in the intraorbital and Harderian glands, while the groups were similar regarding the polymorphonuclear cells and lymphocytes in all the glands. This can be explained by the earlier attenuation of polymorphonuclear and lymphocytic reactions, rather than from the plasmocytic reaction. Since our study evaluates the late (third month) effects of RAI and montelukast, the only conclusion that we can make is that at the third month the groups are similar regarding the polymorphonuclear and lymphocytic reactions, since the early effects are required to be identified. The plasma cells were significantly lower in the ML group, which can be explained by the longer duration of the plasmocytic reaction that is still evident at the third month and diminished by montelukast.

Although the anti-oxidative effect of ML in various conditions has been studied widely, its radioprotective effect has not been studied so far. Our study is the first to report the radioprotective effect of ML on lacrimal glands. ML presents the advantage of possessing anti-oxidant and anti-inflammatory actions together. Thus, both the oxidant and inflammatory components of the radioiodine-related unintended tissue damage are thought to be halted.

The possible role of leukotrienes in the RAI-induced lacrimal gland or any other tissue needs to be evaluated. The effect of ML on early post-RAI inflammation also must be clarified. Clinical studies are needed to identify the potential benefit of ML as a radioprotective agent.

Conclusion

Oral 131I (111 MBq) caused atrophic and fibrotic changes in rat lacrimal glands at the third month. As all the thyroid glands of rats that were treated with RAI were found to be atrophied and no pathologically detectable thyroid tissue remained, ML appears to protect lacrimal glands from the harmful effects of RAI without preventing ablation of the thyroid gland.

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Conflict of interest

The authors declare that they have no conflict of interest.

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