Effects of oral glutamine on radiation-induced laryngeal mucosal damage

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ABSTRACT

Purpose: To evaluate the influence of glutamine on radiation-induced laryngeal mucosal damage in an experimental model.

Methods: The study was conducted at the animal care facility of Istanbul University Institute of Experimental Medicine. Sixteen adult Spraque Dawley rats weighing 200–220 g were used for this study. Radiotherapy was delivered with a 6 MV linear accelerator with two opposed lateral fields. 2000 cGy was given with single fraction to the neck. The animals were randomized into two groups of 8 rats each. Animals in Group I (control group) was fed with standard chow and water, those in Group II was fed with glutamine 2 g/kg/day additional to standard chow and water. All the animals were sacrificed two weeks later with a barbiturate overdose. The larynx was removed and histopathological changes were measured in laryngeal mucosa.

Results: Subepithelial edema and keratosis was higher in Group I than Group II (the p values was 0.012 and 0.034 respectively). Vascular engorgement was higher in Group I but not statistically significant (p: 0.105). There was a significant difference in cartilage degeneration developed in rats receiving standard diet compared to those received glutamine (p = 0.001).

Intraepithelial lymphocyte (IEL) infiltration and inflammation was higher in Group I versus Group II but not statistically significant (p: 0.085 vs. p:0.157 respectively).

Conclusion: Glutamine can accelerate mucosal healing and regeneration in laryngeal mucosa after experimental head and neck irradiation.
INTRODUCTION

Larynx cancer accounts for about one third of all head and neck neoplasms.\textsuperscript{1}

Radiotherapy plays an important role in the treatment of larynx cancer and it is associated with a variety of side effects. Mucosal damage still remains a troublesome problem.\textsuperscript{2}

Efforts on reducing oral mucositis during radiotherapy has been a hot topic for many published clinical investigations.\textsuperscript{3,4,5}

Unfortunately there is few clinical trials investigated laryngeal mucosal damage during radiotherapy. The most common mucosal effect is laryngeal edema. Mucosal damage after radiotherapy is due to impaired vascular and lymphatic flow caused by endothelial damage and fibrosis.\textsuperscript{6}

There are no established measures to prevent radiation induced mucosal damage in humans. Many investigations attempting to reduce mucosal damage have been reported.

Numerous experimental and clinical studies have shown that oral/enteral or intravenous glutamine supports metabolism of the tumor-bearing host and can ameliorate gastrointestinal toxicity of antineoplastic interventions. In the great majority of these clinical studies, glutamine supplementation in cancer patients improves host metabolism and clinical situation. Potential mechanisms of glutamine effects include maintenance of mucosal integrity, improved immune competence, inhibition of cell proliferation, increased apoptosis rate, increased synthesis of glutathione, induction of heat shock protein synthesis, and increased synthesis of glucagons-like peptides.

Glutamine is the most abundant free amino acid in blood, and constitutes for 60% of the total free amino acid pool in skeletal muscle.\textsuperscript{7} Glutamine is a neutral and “nitrogen rich” amino acid that acts as a substrate for nucleotide synthesis in most dividing cells. It contains two amine groups per molecule. It has a critical role as a nitrogen transporter, providing precursor nitrogen for the synthesis of purines and pyrimidines. The significance of glutamine to metabolic homeostasis becomes evident during periods of stress, when it becomes a conditionally essential amino acid.\textsuperscript{8} Concentrations of glutamine in plasma, muscle, and mucosa are reduced significantly after injury, sepsis, and nutritional depletion in humans.\textsuperscript{9,10} During radiotherapy glutamine concentrations in plasma is reduced because of mucosal damage and nutritional depletion.

Glutamine is known as the most important energy source of enterocytes which also lowers the rate of endotoxemia and translocation by preserving mucosal integrity.\textsuperscript{11}

Glutamine is important in the preservation of mucosal structure after radiotherapy. Lack of glutamine may contribute to mucosal damage. Glutamine appears to be beneficial when it is provided after the radiotherapy.

The extent of normal tissue damage from radiation may be influenced by the presence of adequate tissue glutamine stores. These facts support a possible therapeutic role for glutamine in the prevention of host normal tissue toxicity during
radiotherapy. The aim of our study is to discuss the role of glutamine on the laryngeal mucosal damage and the use of supplemental glutamine to prevent specific toxicities related to irradiation.

ETHICS
The study was approved by the Animal Ethics Committee of the Istanbul University School of Medicine, 2012.

ANIMALS AND EXPERIMENTAL DESIGN
Sixteen adult male spraque dawley rats weighing 200–220 g were used for the study. They were housed under constant temperature (22°C) and humidity. The rats were scanned with simulation CT scan (Siemens –somatom sprit model). CT datasets were transferred to the treatment planning system (Eclipse, version 10).

Contouring of larynx volumes were created by Radiation oncologist in treatment planning system (TPS). The treatment planning was performed in the eclipse, version 10. For sparing spinal cord from radiation toxicity, two lateral treatment volumes were used (figure 6). 1 cm bolus material was used to make available dose distribution. The photon beam energy was 6MV, using the linear accelerator (UNIQUE, Varian, USA).

The larynx of the rats was evenly irradiated with 2 Gy per minute. 2000 cGy was given with single fraction to the neck. To immobilize the animals during their irradiation, the rats were anesthetized with an intraperitoneal injection of ketamin hydrochloride (40 mg/kg) and xylazine hydrochloride (5 mg/kg). No side effects or complications occurred during the rats’ anesthesia.

The animals were randomized into two groups of 8 rats each. Animals in Groups I was fed with standard chow and water, those in Group II was also fed with standard chow and water plus glutamine 2 g/kg/ day. One rat from Group I died before the final assessment. All animals were sacrificed on the 15th post radiotherapy day with a barbiturate overdose and evaluations of the histological samples from larynx have been performed.

Statistical analysis
All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Il, USA). The results of each of the treatment groups were compared using Chi-Square test. If there was any significance between the groups, the Mann-Whitney U (MWU) test was used for comparison between the two groups. Values were expressed as the mean values or as a percentage. P<0.05 was considered significant.

HYSTOPATHOLOGICAL EXAMINATION
The larynx was removed after the sacrification of animals. All 15 specimens consisted of the larynx were fixed in 10% formaldehyde, blocked with paraffin and sections of 4–5 micron in thickness were taken. The histopathological changes examined were chronic inflammation in the lamina propria and epithelium, and subepithelial edema, vascular engorgement, keratinization and cartilage destruction.
To evaluate inflammation in the lamina propria and epithelium at magnification of _400, the number of lymphocytes presented at the submucosa was scored as follows: (table 1)

<table>
<thead>
<tr>
<th>Score</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0-20 lymphocytes</td>
</tr>
<tr>
<td>1</td>
<td>21-50 lymphocytes</td>
</tr>
<tr>
<td>2</td>
<td>51-80 lymphocytes</td>
</tr>
<tr>
<td>3</td>
<td>81-120 lymphocytes</td>
</tr>
<tr>
<td>4</td>
<td>&gt;120 lymphocytes</td>
</tr>
</tbody>
</table>

Table 1

and/or, at magnification of _400, the number of intraepithelial lymphocytes was scored as follows: (table 2)

<table>
<thead>
<tr>
<th>Score</th>
<th>Intraepithelial lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>1 or 2 PMNs</td>
</tr>
<tr>
<td>2</td>
<td>3-10 PMNs</td>
</tr>
</tbody>
</table>

Table 2

To evaluate subepithelial edema, vascular engorgement, and keratinization at a magnification of _100, a score was assigned qualitatively according to the degree of subepithelial edema, vascular engorgement, and keratinization: (table 3)

<table>
<thead>
<tr>
<th>Score</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td>3</td>
<td>Marked</td>
</tr>
</tbody>
</table>

Table 3

The presence of regenerative atypia and cartilage destruction were also examined as yes/no.

RESULTS

According to pathological comparison between groups; edema and keratosis was higher in group 1 than group 2. (p:0.012 and p:0.034 respectively). Vascular engorgement was higher in group 1 than group 2, but not statistically significant (p: 0.105). Subepithelial edema and vascular engorgement was seen in laryngeal
mucosa (figure 1-2). There was a significant difference in cartilage degeneration developed in the rats receiving standard diet compared with those which received glutamine \((p = 0.001)\) (Table 4).

**Table 4.** Cartilage degeneration distribution in groups.*

<table>
<thead>
<tr>
<th>Cartilage degeneration</th>
<th>No Cartilage degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>n(%)</td>
<td>n(%)</td>
</tr>
<tr>
<td>Group 1</td>
<td>1( 14.3)</td>
</tr>
<tr>
<td>Group 2</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

*: Analyses were made using Chi-Square test. \(P: 0.001\)

Intraepithelial lymphocyte (IEL) infiltration and inflammation was higher in group 1 versus group 2 but not statistically significant \((p : 0.085 \text{ and } p:0.157 \text{ respectively})\) (Table 5).

**Table 5.** Pathological comparison between groups*

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>3.86 ± 0.38</td>
<td>3.50 ± 0.53</td>
<td>0.157</td>
</tr>
<tr>
<td>IEL</td>
<td>1.86 ± 0.90</td>
<td>2.63 ± 0.74</td>
<td>0.085</td>
</tr>
<tr>
<td>Vasc. Eng.</td>
<td>1.71 ± 0.76</td>
<td>1.00 ± 0.76</td>
<td>0.105</td>
</tr>
<tr>
<td>Edema</td>
<td>1.43 ± 0.53</td>
<td>0.38 ± 0.74</td>
<td>0.012</td>
</tr>
<tr>
<td>Keratose</td>
<td>2.43 ± 1.13</td>
<td>1.75 ± 0.46</td>
<td>0.034</td>
</tr>
</tbody>
</table>

*: Analyses were made using Mann-Whitney U test

Severe lymphocytic (score 4) and polymorphonuclear leukocytic (score 3) infiltration was seen in the lamina propria (Figure 3). Score 3 intraepithelial lymphocytes was seen in the epithelium (Figure 4).

Score 3 polymorphonuclear leukocytic infiltration was seen in lamina propria (Figure 5).

**DISCUSSION**

Radiation mucositis is considered to be an inevitable but transient side-effect of therapeutic head and neck irradiation.\(^{12}\) Prevention of mucosal damage due to radiation is still limited to reduction of its severity by oral care programs.

There have been many agents investigated to prevent mucosal damage due to therapeutic irradiation. Sucralfate is one of them and it has been used to treat gastric ulcers and requires an acid environment to be activated. Based on the mucosa-
coating effect of sucralfate which may promote recovery, there have been attempts to
improve the mucosal damage caused by radiotherapy or chemotherapy. In one trial
there was no clinical evidence indicating that the oral intake of sucralfate reduces the
acute radiation-induced mucosal damage (5) Meredith et al. also showed the same
result with sucralfate.13 Another agent is corticostearoid and Leborgne et al.
concluded that the corticosteroids also did not reduce the intensity or duration of
mucosal damage but could shorten treatment interruption in a placebo-controlled
trial.14 Topical anesthetics such as xylocaine (lidocaine) has been proposed for oral
mucositis. There is also research indicating that the administration of growth factors
(granulocyte-macrophage colony-stimulating factor, keratinocyte growth factor) has a
potential to reduce the development of oral mucositis and larengeal mucosal damage
and can significantly promote healing.15,16

Especially last 30 years a vast experimental and clinical data mounted regarding
 glutamine usage under some special clinical conditions like oncology.

In animal models, enteral glutamine has been shown to be effective in reducing the
mucosal damage associated with chemotherapy induced enteritis.17, 18 Enteral
 glutamine supplementation yields a better survival rate than parenteral
supplementation when it is administered after chemotherapy or radiotherapy.19

In one randomized trial of 17 patients who were receiving radiation for head and neck
cancer, those who were randomized to oral glutamine (2 g, four times daily during
radiation) had a significantly shorter duration of objective oral mucositis, less severe
maximum grade of mucositis, than did the placebo group.20

It has been shown to be effective in reducing the severity of radiation-induced
mucosal injury of bowel in rats.21, 22

Glutamine supplementation of an elemental diet resulted in less weight loss,
increased mucosal weight of the jejunum and colon, longer survival, and a lower
incidence of bacteremia among rats treated with chemotherapy.17, 23

Thus, glutamine plays an important role in the preservation of intestinal mucosa
integrity after radiotherapy and chemotherapy. Oral glutamine has prevented
chemotherapy-induced stomatitis in some recent studies.24,25

But, there is still no data suggesting a possible protective effect of oral glutamine in
radiation-induced laryngeal mucosal damage.

**Could glutamine be effective on laryngeal mucosal tissue damage induced by
radiotherapy?**

There are no related reports about the influence of glutamine on radiation-induced
oral mucositis or laryngeal mucosal damage. In order to acquire some hints and shed
some lights on the topic we conducted a study of oral glutamine given after
radiotherapy in an experimental model. We investigated acute and chronic effects on
laryngeal mucosa such as inflammation, intraepithelial lymphocyte (IEL) infiltration,
edema, vascular engorgement and keratosis with supplemented glutamine.

Radiotherapy was delivered as a single dose to limit anesthesia-induced death. The
rats were sacrificed 15 days later because it has been reported that acute side
effects develop within 2 weeks of irradiation, while chronic side effects start about at 2 weeks.\textsuperscript{26}

20 Gy dose was chosen for this experiment because irradiation of 20 Gy to rats is the biological dose equivalent of irradiation of 60 Gy to humans.\textsuperscript{27, 28}

In this experimental study, the rats which were fed with glutamine 15 days after radiotherapy had less severe mucosal damage than control group.

CONCLUSIONS

Glutamine is a molecule that influences body homeostasis. Supplementation with this dietary nutrient may have an important role in the prevention of laryngeal mucosal damage of radiotherapy. This complication often negatively affects quality of life and may also lead to changes in the course of therapy, which potentially alter efficacy. Further studies with glutamine supplementation in this area are warranted.

We concluded that the glutamine supplemented diet is superior to standard diet when it is given with head and neck radiotherapy related of mucosal damages.

References:


