The Effect of Rat Tympanic Membrane Perforation on Oxidants/Antioxidants Balance

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Abstract. Myringosclerosis is a common sequel of the ventilation tube treatment of otitis media. Recent studies have established the relationship between reactive oxygen species (ROS) and myringosclerosis. The aim of this experimental study was to investigate the temporal change of oxidants/antioxidants balance at 6, 12, 24, and 48 hours after the myringotomy.

Fifty healthy adult male Wistar albino rats, weighing approximately 250-300 g, were randomly separated into five groups (n = 10 rats/group): first group, of control, nonmyringotimized and four groups myringotimized unilaterally on the left ear.

The tympanic membranes were collected from sacrificed rats for biochemical evaluation of parameters of oxidants/antioxidants balance from 1st group and in 6 hours from 2nd group, in 12 hours from 3rd group, in 24 hours from 4th group and in 48 hours from 5th group.

The index oxidants were malondialdehyde and carbonyl proteins and the index antioxidants were hydrogen donor ability and sulfhydryl groups.

Results and conclusions: The changes of oxidants/antioxidants balance and oxidative stress appear after myringotomy in 6 hours and incease in 12, 24, and 48 hours. Myringosclerosis develops promptly after myringotomy.

Key words: myringotomy, myringosclerosis, rat, tympanic membrane, oxidative stress, antioxidants.

INTRODUCTION

Myringosclerosis is a common sequel of the ventilation tube treatment of otitis media. Recent studies have established the relationship between reactive oxygen species (ROS) and myringosclerosis. The aim of this experimental study was to investigate the tissue change of oxidants/antioxidants balance (O/AO balance) at 6, 12, 24, and 48 hours after the myringotomy.

MATERIALS AND METHODS

The researches were performed in white male Wistar rats from the Biobase of the “Iuliu Hațieganu” University of Medicine and Pharmacy Cluj-Napoca, with a weight of 250-300 g, maintained under adequate vivarium conditions, at the Biobase of the Department of Physiology.

a. Groups
The animals were assigned to 5 groups of 10 rats each:

• group I – control group – myringotomized rats, sacrificed initially
• group II – myringotomized rats, sacrificed after 6 hours
• group III – myringotomized rats, sacrificed after 12 hours
• group IV – myringotomized rats, sacrificed after 24 hours
• group V – myringotomized rats, sacrificed after 48 hours

The animals were euthanized and then sacrificed.

Myringotomy was performed in the Laboratory of Experimental Researches of the Department of Physiology of the “Iuliu Hațeganu” University of Medicine and Pharmacy, Cluj-Napoca. The operative procedure used was adapted from Mattsson et al. 1997 and Polat et al. 2004.

Tissue samples were collected from the tympanic membrane of the middle ear for biochemical determinations of the indicators of the O/AO balance, performed in the Laboratory for the Study of OS of the Department of Physiology.

b. Biochemical methods
The indicators of the O/AO balance from the tissue homogenates of the middle ear were determined:
- oxidative indicators: malondialdehyde (MDA) (fluorescence method, according to Conti), carbonylated proteins (CP) (dosage method according to Reznick);
- antioxidant indicators: hydrogen donor ability (HD) (dosage method according to Janaszewska), sulphydryl groups (SH) (dosage method according to Hu).

c. Statistical processing
For each set of values, descriptive statistical elements were calculated; for the statistical analysis of data of the 4 groups, in the case of normal distribution data, the Student test was used for unpaired samples.

In the case of unevenly distributed values, the Student test was replaced by the non-parametric tests Kruskal-Wallis – for 3 or more independent samples and Mann-Whitney (U) – for 2 independent samples.

Statistical processing was performed using the Excel application (Microsoft Office 2003), with the SPSS v.16 software or online, using the OpenEpi v.2.2.1 application.

RESULTS

a. The oxidants/antioxidants balance

The indicators of the O/AO balance from the tympanic membrane tissue homogenate are shown in Table I.
Table 1
Indicators of the oxidants/antioxidants balance in the studied groups

<table>
<thead>
<tr>
<th>Tissue homogenate</th>
<th>Malondialdehyde (MDA)</th>
<th>Carbonyl Proteins (CP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Mean</td>
<td>0.603</td>
<td>1.320</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.09280</td>
<td>0.01238</td>
</tr>
<tr>
<td>Median</td>
<td>0.58</td>
<td>1.32</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.29345</td>
<td>0.03916</td>
</tr>
<tr>
<td>Sample Variance</td>
<td>0.8611</td>
<td>0.00153</td>
</tr>
<tr>
<td>Range</td>
<td>0.910</td>
<td>0.130</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.290</td>
<td>1.350</td>
</tr>
<tr>
<td>Maximum</td>
<td>1.200</td>
<td>1.380</td>
</tr>
<tr>
<td>Count</td>
<td>6.03</td>
<td>13.2</td>
</tr>
<tr>
<td>Confidence Level</td>
<td>0.20992</td>
<td>0.02801</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue homogenate</th>
<th>Hydrogen Donors (HD)</th>
<th>Sulfhydryl Groups (-SH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Mean</td>
<td>44.800</td>
<td>22.400</td>
</tr>
<tr>
<td>Standard Error</td>
<td>2.2944</td>
<td>0.03915</td>
</tr>
<tr>
<td>Median</td>
<td>46.35</td>
<td>22.40</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>7.25565</td>
<td>0.11116</td>
</tr>
<tr>
<td>Sample Variance</td>
<td>52.6444</td>
<td>0.01238</td>
</tr>
<tr>
<td>Range</td>
<td>22.120</td>
<td>0.400</td>
</tr>
<tr>
<td>Minimum</td>
<td>32.500</td>
<td>22.200</td>
</tr>
<tr>
<td>Maximum</td>
<td>54.600</td>
<td>22.600</td>
</tr>
<tr>
<td>Count</td>
<td>448</td>
<td>224</td>
</tr>
<tr>
<td>Confidence Level</td>
<td>5.19356</td>
<td>0.07952</td>
</tr>
</tbody>
</table>

Our results indicate:

• for MDA
  o highly significantly lower values (p < 0.001) in group I compared to groups II, III, IV and V, in group II compared to groups III, IV and V and in group III compared to group V
  o significantly lower values (p < 0.05) in group III compared to group IV
  o insignificant differences between groups IV – V

• for CP
  o highly significantly lower values (p < 0.001) in group I compared to groups II, III, IV and V, in group II compared to groups III, IV and V and in group III compared to groups IV and V
  o insignificant differences between groups IV – V

• for HD
  o highly significantly higher values (p < 0.001) in group I compared to groups II, III and IV
  o highly significantly lower values (p < 0.001) in group II compared to groups III, IV and V, in group III compared to groups IV and V and in group IV compared to group V
  o insignificant differences between groups I – V

• for SH groups
  o highly significant differences (p < 0.001) between the 5 groups
  o highly significant differences (p < 0.001) between groups I – II, I – III, II – IV, II – V, III – V and IV – V
  o insignificant differences between groups I – V, II – III, II – IV and III – IV

b. Correlation between the indicators of the oxidants/antioxidants balance

Table II shows Pearson r correlation coefficients between MDA, CP, SH and HD in the studied groups.
Table 2
Bravais-Pearson correlation coefficients between MDA, CP, HD and SH determined from the tissue homogenate in the studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA-PC Correlation</th>
<th>MDA-DH Correlation</th>
<th>MDA-SH Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.5003 ***</td>
<td>0.0420 *</td>
<td>0.2454 *</td>
</tr>
<tr>
<td>Group II</td>
<td>0.7634 ****</td>
<td>-0.3472 **</td>
<td>-0.1543 *</td>
</tr>
<tr>
<td>Group III</td>
<td>0.3333 **</td>
<td>0.3333 **</td>
<td>0.7782 ****</td>
</tr>
<tr>
<td>Group IV</td>
<td>-0.2614 **</td>
<td>-0.2603 **</td>
<td>-0.0616 *</td>
</tr>
<tr>
<td>Group V</td>
<td>-0.2228 *</td>
<td>-0.1061 *</td>
<td>0.1387 *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PC-DH Correlation</th>
<th>PC-SH Correlation</th>
<th>DH-SH Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>-0.3020 **</td>
<td>-0.1357 *</td>
</tr>
<tr>
<td>Group II</td>
<td>-0.1503 *</td>
<td>-0.0481 *</td>
</tr>
<tr>
<td>Group III</td>
<td>1.0000 ****</td>
<td>0.3374 **</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.9996 ****</td>
<td>0.3374 **</td>
</tr>
<tr>
<td>Group V</td>
<td>-0.1435 *</td>
<td>0.1178 *</td>
</tr>
</tbody>
</table>

* = Weak/null correlation
** = Acceptable correlation
*** = Moderate/Good Correlation
**** = High Correlation

The study showed the following:

- for group I
  - the absence of a significant correlation between MDA – HD, MDA – SH and CP – SH
  - an acceptable negative correlation between CP – HD
  - a good positive correlation between MDA – CP and HD – SH

- for group II
  - the absence of a significant correlation between MDA – SH, CP – HD and CP – SH
  - an acceptable negative correlation between MDA – HD and HD – SH
  - a very good positive correlation between MDA – CP

- for group III
  - an acceptable positive correlation between MDA – CP, MDA – HD, CP – SH and HD – SH
  - a very good positive correlation between MDA – SH and CP – HD

- for group IV
  - the absence of a significant correlation between MDA – SH
  - an acceptable negative correlation between MDA – CP and MDA – HD
  - an acceptable positive correlation between CP – SH and HD – SH
  - a very good positive correlation between CP – HD

- for group V
DISCUSSION

Myringotomy is one of the most frequent surgical procedures used in pediatric treatment in patients with acute otitis media (AOM) and secretory otitis media (SOM), with or without the insertion of a ventilation tube.

Until the 18th century, myringotomy was used for the treatment of deafness. Experimentally, the procedure was initially used in the tympanic membrane of rats, mice, hamsters, guinea pigs and cats, with a structure similar to that of humans (Spratley et al. 2002).

$O_2$ concentration in the middle ear is decreased to values of about 5-10% compared to atmospheric air. Gases are in equilibrium with blood gases or with their partial pressure in the mucosae. The perforation of the tympanic membrane with the increase of oxygenation in the middle ear occurs rapidly, with the creation of non-physiological conditions and changes in $O_2$ metabolism. Mass spectroscopy evidenced $O_2$ values of 9.2% in the controls (for pressures of 713 mm Hg), while patients with exudative otitis had $O_2$ values of 15.6% and those with chronic perforated otitis media, $O_2$ values of 16.9% (Okubo et al. 1994). With the perforation of the tympanic membrane by myringotomy, the increase in reactive oxygen species (ROS) is determined by hyperoxic conditions as well as by the activation of phagocytic cells and inflammation (Mattsson et al. 1998).

Our results are in accordance with the data of Polat et al. (2004), who measured ROS levels by chemiluminiscence in myringotomized tympanic membranes, and with the experimental data of Mattsson et al. (1999), based on optical and electron microscopy, regarding the development of myringosclerosis in the pars flaccida 9 hours after myringotomy and in the pars tensa 24 hours post-myringotomy. The increase in $O_2$ concentrations in the middle ear and relative hyperoxia through myringotomy favor an increase in tissue oxygenation with an increased ROS production (Buckingam et al. 1985, Felding et al. 1987, Mover-Lev and Sade 1998).

Our researches demonstrated through the direct measurement by biochemical methods of MDA and CP levels in the tympanic tissue that OS appears early after myringotomy, with an increase in ROS levels and a decrease in the AO defense capacity.

CONCLUSIONS

1. Changes in the O/AO balance occur promptly after myringotomy, at 6 hours, with an increase in OS (MDA and CP) and a decrease in AO defense (HD and SH groups).
2. OS intensity increases post-myringotomy, reaches the highest values at 24 hours, and is maintained at 48 hours.
3. Post-myringotomy AO defense is maintained low at 6-12-24 hours, with an increasing tendency at 48 hours.
4. Myringosclerosis develops promptly after myringotomy and is in relation to OS.
REFERENCES


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