The purpose of this study was to determine whether there is a correlation between induced occlusal trauma and the occurrence of oxidative stress in the hippocampus. Twenty Wistar rats were randomized into three experimental groups and one control group. Animals in the experimental group were cemented modified crowns on molars to induce occlusal trauma in 7, 14 and 30 days. To evaluate the oxidative stress, blood was drawn from the hippocampus at 7, 14 and 30 day intervals. Oxidative stress was evaluated using the following specific tests: determination of plasma malondialdehyde and serum glutathione values. The results of the study demonstrate that malocclusion experienced by raising the vertical dimension in rats resulted in significant reductions in antioxidants and increase level of free radicals.

Keywords: occlusal trauma, hippocampus, free radicals
type II L polycarbonate cages, in a room with a constant
temperature (21.5–23 degrees) and 65% relative humidity.
The animals were exposed to a standard 12 hours light/
dark cycle. The rats were administered a light diet and
water ad libitum.

The research protocol was approved by the Ethics
Committee of UMPh Cluj-Napoca and was registered under
the number 39/16.

The animals were randomized to four groups as follows:
1- control group
2 -group with occlusal trauma (OT) for a 7-day period
3 - group with occlusal trauma (OT) for a 30-day period
4 -group with occlusal trauma (OT) for a 30-day period

Occlusal trauma was induced by placement of 0.5 mm
thick Ni-Cr metal crowns on the mandibular first molar of
the right quadrant in 15 animals. The 0.5 mm thick metal
crowns were made in the dental laboratory, taking into
consideration the small size of dental crowns for
experimental animals. The rats were anesthetized with
intramuscular Ketamine 0.3 mg/g body weight (b. w) and
Narcoyl 0.1 mg/g b. w. Before placement of the metal
crown, the occlusal surface was professionally brushed.
The crowns were cemented with dual cement (BisCem).

Evaluation of oxidative stress

To evaluate the oxidative stress, blood was drawn from
the hippocampus at 7, 14 and 30 day intervals. Oxidative
stress was evaluated using the following specific tests:
determination of plasma malondialdehyde (MDA) and
serum glutathione (GSH) values.

Method of dosing malondialdehyde by fluorescence

The determination of lipid peroxidation by fluorescence
is based on the fact that the malondialdehyde resulting
from this process forms a fluorescent adduct with the
thiobarbituric acid. For dosing, the plasma sample or tissue
homogenate is boiled for 1 hour with a solution of 10 mM
2-thiobarbituric acid in K2HPO4, 75 mM and pH 3. After
rapid cooling, the reaction product is extracted into n-
butanol. Its concentration is determined in the organic
phase after separation by centrifugation. Measurement of
emission intensity was done at 534 nm with a Perkin Elmer
spectrofluorimeter, by a synchronous fluorescence
emission intensity  was done at 534 nm with a Perkin Elmer
spectrofluorimeter, by a synchronous fluorescence
emission technique with a wavelength difference of 14 nm between
excitation and emission (∆λ).

The concentration of
malondialdehyde was determined on the basis of a
calibration curve made with known concentrations of MDA,
processed in the same way. The concentration values are
expressed in nmol/mL [13].

After 15 min, the emission intensity is measured at 420
nm at an excitation of 350 nm.

The concentration of glutathione is determined using a
calibration curve made with known concentrations of
 glutathione processed in the same manner. The
concentration values are expressed in nmol/mL [14].

The statistical analysis of the results was performed
using the Anova test for repeated measurements. All values
obtained are expressed as mean and standard deviation
of the mean. The level of statistical significance was p <0.05
.

Results and discussions

First of all, we attempted to identify the metallic
prosthesis used for the creation of the occlusal trauma, by
checking whether the surgical technique was performed
correctly or not. We noticed that the metal prosthesis was
correctly cemented in all the animals under study. The
crowns were cemented with BisCem, which is a self-
etching, self-adhesive, dual-cured resin.

Composite luting cements are very important for
adhesive cementation in fixed prosthodontics [15].
Adhesive cements are unique due to the achievement of
adhesive bonding for both interfaces: dental tissue and
restoration material. These cements have been chemically
modified to have high shear bond strength and
simultaneously adhere to etched dental tissues and metal
restorations, which can be noble and non-noble,
electrolytic etched or sandblasted [16].

Analyzing specific oxidative stress, we noticed a
connection between occlusal trauma, free radical increase,
and antioxidant decrease. The study demonstrated that
the GSH antioxidant alters its plasma value according to
the duration of exposure to occlusal trauma. The results
showed that the animals in group 4 had the lowest values
when compared to the previous groups 1, 2 and 3.

The GSH antioxidant showed a decrease in plasma
levels in the experimental animals in comparison to the
control group. For group 2 (OT = 7 days), an average of
0.51536500 and p <0.02 were recorded. At 14 days after
the experiment, for group 3, the mean was 0.13718620
and p <0.037 (table 1).

At the hippocampus level, plasma levels of MDA showed
significant differences. Group 2 (OT = 7 days) showed an
average of 0.07724240 and p <0.02. Plasma values
increased to 0.11151680 after 14 days for group 3. After 30
days, the mean MDA was 0.19346560 and p <0.05 for

Table 1 (No.12 2018)

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>OT = 7 days</th>
<th>OT = 14 days</th>
<th>OT = 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>0.37454460</td>
<td>0.13718620</td>
<td>0.11151680</td>
<td>0.19346560</td>
</tr>
<tr>
<td>GSH</td>
<td>0.151283962</td>
<td>0.126588550</td>
<td>0.072109655</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.019</td>
<td>0.011</td>
<td>0.037</td>
<td></td>
</tr>
</tbody>
</table>
nervous system are rich in polyunsaturated fatty acids, which constitute the potential target of lipid peroxidation [8]. Increased oxidative action under the influence of occlusal trauma may result from an increase in the production of reactive oxygen species or from a reduction in oxidative capacity.

Exposure to chronic stress affects the survival, proliferation and differentiation rate of young cells. Stress is associated with elevated glucocorticoid levels leading to apoptosis [17]. The presence of glucocorticoids in elevated concentrations decrease the antioxidant defense capacity in neurons. Thus, the current study also reported the decrease in peroxidase glutathione in the hippocampus. Plasma GSH values have been continuously decreasing from the first group to the end of the 30-day experiment.

Statistically significant differences between the experimental groups and the control group with regard to the GSH values suggest the existence of oxidative stress and the appearance of free radicals.

Conclusions

The results of the current study demonstrate that malocclusion experienced by raising the vertical dimension in rats resulted in significant reductions in antioxidants and increase in free radicals. Additional studies are required in order to clarify the precise mechanisms by which occlusal disharmony exerts its effects on the function of the hippocampus.

References


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