Acute pulpitis as a cause for the expression of inflammatory markers in the amygdala of mice

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Abstract

Background and aims

Pulpitis is an acute clinical condition characterized by a series of immune and inflammatory events initiated by the pulp tissue in an attempt to contain the progression of injuries that compromise local homeostasis (Al Natour et al., 2021). The interaction between nociceptive terminals within the pulp tissue and resident or recruited immune cells is fundamental for protecting the dentin-pulp complex and regulating inflammatory responses (Pinho-Ribeiro, et al., 2017). The persistence of inflammatory conditions, especially those associated with pain, can be related to alterations in brain areas related to stress regulation (Spinieli et al., 2022). Given the clinical presentation, particularly in the case of acute pulpitis, with a high frequency of pain perception, it is important to investigate this relationship. Thus, the aim of this study is to evaluate inflammatory aspects at both the local and central levels during the development of experimentally induced pulpitis in mice.

Methods

This study was approved by the Ethics Committee on Animal Use (231.1.395.59.0). A total of 48 wild-type isogenic mice of the *Mus musculus* (C57BL/6) were used. Mice were divided in two groups: G1- experimental group (n=24) and G2 - control group (n=24). For pulpitis induction, the animals were anesthetized, and the coronal pulp of molar was accessed using a 1/4 drill. A 10 µm solution of LPS (1 µg/µL) was inoculated, and the

access was sealed using glass ionomer. Euthanasia was scheduled on days 2, 5, and 10 post pulp access, for each group (n=4). After euthanasia, mice had their right mandibles collected for histomorphological evaluation of local inflammation. Additionally, mice brains were collected for relative quantification of nuclear factor κB (NF-κB) and Glial Fibrillary Acidic Protein (GFAP) to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the amygdala complex using Western Blotting technique. Data were analyzed using ANOVA two-way, followed by Tukey's post-test ($p<0.05$). Central and local inflammation data were also tested by Pearson and Sperman correlation ($p<0.05$).

Results

The two-way ANOVA test indicated a statistically significant difference in the count of mononuclear inflammatory cells and polymorphonuclear only concerning the group (experimental and pulpitis) ($p<0.05$). No significant difference was observed between the experimental days (days 2, 5, and 10) ($p>0.05$). Areas of necrosis, inflammatory exudate, and hyperemia were qualitatively identified and described by scores, with no statistically significant association with the groups (G1 and G2) or the experimental days (days 2, 5, and 10) ($p<0.05$). The Western Blotting assay showed results with a statistically significant alteration in the relative levels of NF- κB in the amygdala complex of mice between the experimental group and the control group ($p<0.05$), but without a difference between the experimental days ($p>0.05$). Regarding GFAP levels, statistically significant differences were identified between the experimental and control groups ($p<0.05$) but not between the experimental days ($p>0.05$). In the correlation analysis, NF-kB relative expression showed positive and moderate correlation to the inflammatory cells count ($p<0.05$).

Conclusions

According to the employed methods, it was possible to identify acute local inflammation at all post-pulpal injury time points. Clear characteristics, such as an abundance of polymorphonuclear cells, presence of exudate, hyperemia, and even areas of necrosis near the exposure site, were histologically identified. Additionally, concerning the central level of inflammation, a significantly important expression of NF- κB in the amygdala complex

was observed. These findings support the initial hypothesis of the potential of acute pulpitis to centrally sensitize the individual. Future studies should aim to associate these findings with behavioral changes, alterations in peripheral sensitivity threshold, nociceptive changes, and investigate the involvement of other inflammatory markers in this context.

References

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Relevance for patient care

The execution of this study has provided clarification on the role of acute pulpitis as a factor in central sensibilization in individuals. Understanding these common occurrences of local and central inflammation caused by a same local pathological condition, with a considerable suffering involved, such as the pulpitis is crucial to better understanding of the clinical context. Animal models are a great option to conjugate factors and comprehend the potential associations and correlations, based on clinical observations, in a translational way. Pulpitis as a causal factor for amygdala inflammation is an important find, and should be further explored searching for behavioral changes, sensitivity alterations and nociception and pain modulation. This set will be plenty useful for clinical management changes and to achieve new therapeutic approaches.