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Critical considerations when planning experimental *in vivo* studies in dental traumatology

REVIEW ARTICLE

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Abstract – *In vivo* studies are sometimes needed to understand healing processes after trauma. For several reasons, not the least ethical, such studies have to be carefully planned and important considerations have to be taken into account about suitability of the experimental model, sample size and optimizing the accuracy of the analysis. Several manuscripts of *in vivo* studies are submitted for publication to *Dental Traumatology* and rejected because of inadequate design, methodology or insufficient documentation of the results. The authors have substantial experience in experimental *in vivo* studies of tissue healing in dental traumatology and share their knowledge regarding critical considerations when planning experimental *in vivo* studies.

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The scientific basis for dental traumatology has to a large extent been derived from *in vivo* studies where clinical situations like fractures, luxations, and avulsions with subsequent replantation have been simulated. This naturally poses the question of how reliable are these experiments for the human clinical counterpart situation. Several manuscripts of such studies have been submitted to our journal *Dental Traumatology* and rejected because of inadequate methodology or insufficient documentation of the results. This is very unfortunate, not the least for ethical reasons. The purpose of this article is to describe some of the obstacles in performing animal experimentation for dental trauma studies and necessary documentation for studies. In that regard, the following issues will be discussed:

1 Is the animal model suitable for providing a reliable answer to the problem?
2 Are the number of animals (or teeth) used sufficient to prove or disprove the underlying hypothesis?
3 If histologic examination is part of the study, will the procedure be accurate enough for – the examination (*sensitivity*, i.e. diagnosing pathologic events and *specificity*, i.e. diagnosing healthy tissue conditions) at its optimum
4 How to analyze and report different healing responses?

Animal models - ethical considerations

The first limitation for the researcher to consider before starting experiments on animals is whether one can simulate a trauma situation in an animal. Situations with limited tissue injury may be simulated in experimental models, whereas more extensive injury cannot be simulated for ethical reasons. Animal experiments should always be as humane as possible. It is wrong to use animals if alternative testing methods can produce equally valid results. Moreover, experimenting on animals is acceptable only if suffering is eliminated or limited in all experiments, and advantages are gained which cannot be obtained by using other methods. The number of animals used in experiments should be minimized as much as possible by optimizing data analysis, improving experimental techniques and sharing information with other researchers. The researchers should always continuously refine the experiments through the use of less invasive techniques, better care and better living conditions for the animals. Whenever

possible, animals can be replaced by alternative techniques e.g., *in vitro* experiments on cell cultures instead of whole animals.

There are ethical codes for animal care in scientific experiments and the purpose of the codes are to ensure the ethical and humane care and use of animals used for scientific purposes. These ethical codes vary across different countries all over the world. In some countries, there are very strict rules for animal research and in many countries there are ethical committees specifically for animal research giving their approval and suggestion for modification before an experiment can be started. In some countries, veterinarians are even present during the experiments to take care of the animals. Before you start an experiment in which animals are involved, you must be familiar with the ethical codes of the country where the experiment is carried out. When reporting your results, scientific journals nowadays require a certification from the author that the code of the country where the research has been carried out has been followed when submitting a manuscript.

Animal models for studies on tissue healing

Pulp

Smaller animals like mice, hamsters and rats have been used for pulp healing research in molars; however, the very limited size of their pulp (2–3 mm in length) makes it difficult to create comparable situations to a human pulp (Fig. 1) (1–3). Moreover, their closely positioned incisors are continuously growing (Fig. 1). The Rabbit has been used for pulp studies (4); however, incisors are continuously growing also in these animals and this must be taken into consideration when performing such studies on teeth. Dogs are frequently used and represent for many reasons a better model than mice and rabbits for pulp studies (5–11). A limiting factor here is the apical delta in mature teeth, which makes healing responses difficult to compare to humans (Fig. 2). To overcome this problem, the apex is often resected (before or after extraction) to make a root filling or a revascularization procedure similar to a human

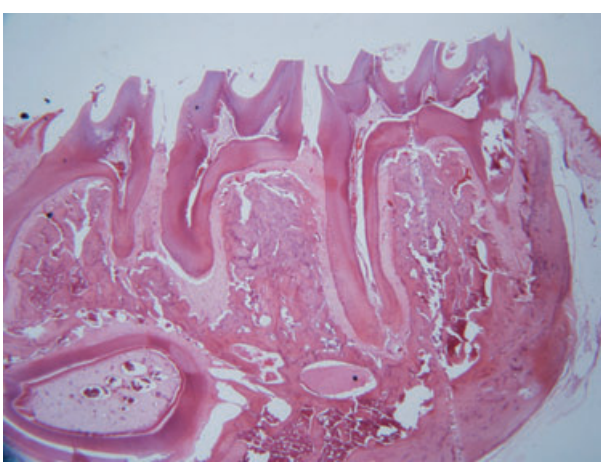

Fig. 1. Longitudinal mesio-distal section of three rat molars, close to the lower border is the apical part of continuous growing incisor seen.

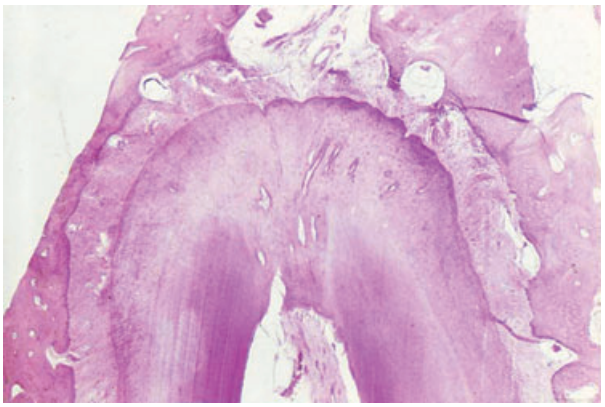

Fig. 2. Closed apex with apical delta in a fully developed dog premolar.

situation. This makes the endodontic procedure rather similar to a human tooth; however, the revascularization experiments seem to overrate the healing rate in comparison with humans because of an extraordinary vigorous healing response in dogs compared with humans (12). To overcome this, swine may be used for pulp studies (13) The monkey model has been widely used for pulp healing studies (exposure, capping, pulpotomy and revascularization) and appears to be rather reliable in all of the above-mentioned situations (14–23).

Periodontal ligament (PDL)

The response of the periodontal ligament in animals when compared to humans can be similar in a few situations but definitely not in others. Some trauma situations may not be simulated, whereas others may be easily simulated. A tooth avulsion situation is typically in animal experimentation replaced by an extraction situation, a scenario that possibly differs somewhat from an avulsion situation, especially with regard the compression damage to the PDL (16, 24). Nevertheless, taking the differences into consideration before drawing conclusions, such models can be used. For species like rabbits and rats, there are significant differences in PDL anatomy that make the healing response to avulsion and replantation less conclusive (1–3). In rats, the PDL response of molars has been examined for trauma and surgical response. However, their intensive bone response to injury makes this model doubtful (1). The most frequent model for avulsion and replantation studies appears to be dogs (9, 25–33) and monkeys with wound-healing responses similar to humans (14, 15, 17, 34–49).

Bone and soft tissue

For studies of healing of bone and oral soft tissue, rabbits have been used (50–53).

Is the number of animals (experimental teeth) sufficient to prove or disprove a hypothesis?

For ethical and practical reasons, the number of animals (teeth) is in most cases very limited. Always

consult a statistician when you plan an experiment. A test is to estimate the outcome of the treatment differences which is considered important (e.g. 10%, 50%) and the level of significance (1%, 5%) to estimate the number of animals (teeth) needed (54). The variable studied is also important, whether the impact of the treatment will be solely on the tooth/cell level or if differences between different animals may have an impact, which will usually require more animals for the experiment.

Will the examination procedure of the healing result be possible to evaluate with a reasonable accuracy?

Pulp healing

Pulp healing can be monitored by electrometric sensibility tests in clinical follow-up studies (55, 56), and laser Doppler (55, 56), all procedures which have been proven to be reasonably accurate in the clinic. Also radiographic examinations have been used (57). However, when studying the healing of tissues, we must rely on experimental animal studies using histologic evaluation. This procedure has a number of pitfalls, which should be eliminated or at least controlled. A number of artefacts may occur during tissue preparation, which simulates pathology (see Langeland 1957)(58). Furthermore, the sectioning direction and number of sections used are very critical (16, 59). Sections along the axis of the tooth are frequently used, and with relatively few sections, one can have a good view of the status of the central part of the pulp (Fig. 3). A more elaborate technique is to use step-serial sections perpendicular to the axis, which gives more precise information about the whole status of the pulp (Fig. 3).

With regard to classifying the extent of pathology, several indices have been presented and should be consulted for their relevance in the particular experimental situation (60).

As most pathology is related to bacterial presence, histo-bacterial stains should be applied when indicated (16).These stains may be technically difficult to perform but appear to be reasonably reliable (16). Lately, PCR techniques have been introduced; however, their accuracy is presently very much debated (61).

PDL responses

The healing state of an injured tooth can be monitored by a variety of methods ranging from *mobility testing* (62), *radiography* (42, 62) to *histology* (7, 16, 26, 40–44). The last method is the most used (and misused). Usually, the experiment has the purpose of comparing PDL healing using one or more treatment interventions. To analyze the healing response in the PDL, it is necessary to realize that for instance, root resorption that attacks on the root is not evenly distributed on the root surface but may have preferences for corner surfaces (16, 59). If a single axial section is used as reference of a 'corner stage' of resorption, one section direction may show 0% resorption, whereas another section direction may demonstrate 100% resorption (Fig. 4) (16, 42, 59). In apico-coronal direction the same irregularity in the presence of root resorption may exist. This calls for a step-serial horizontal section of the root perpendicular to the axis of the root (16, 59). If step-serial sections are performed and a sufficient number of 'reading' locations are defined, the accuracy of a 'pathology index' can be reasonably precise (16, 59) (Fig. 3). The reader is referred to special articles about this problem (16, 59). A method for evaluation of residual root mass after root resorption has been described (7).

Statistical methods to be used in histologic studies

A popular method has been to grade inflammation responses in the pulp using a nominal scale (e.g. 0–3)(63) and the PDL using a 0–100% response of pathology (16). It may be tempting to use means and standard deviations

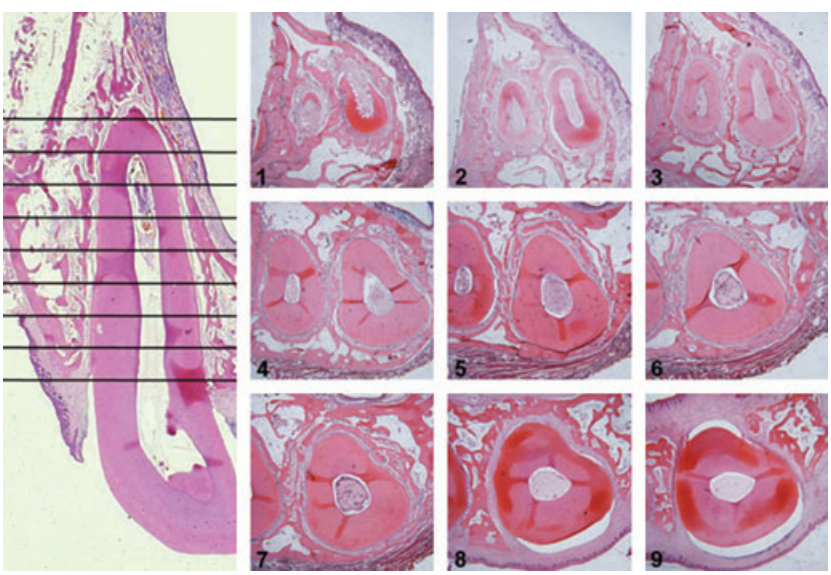

Fig. 3. Longitudinal mesio-distal section of a monkey central incisor compared with horizontal step-serial sections for evaluation of pulp and periodontal responses to trauma.

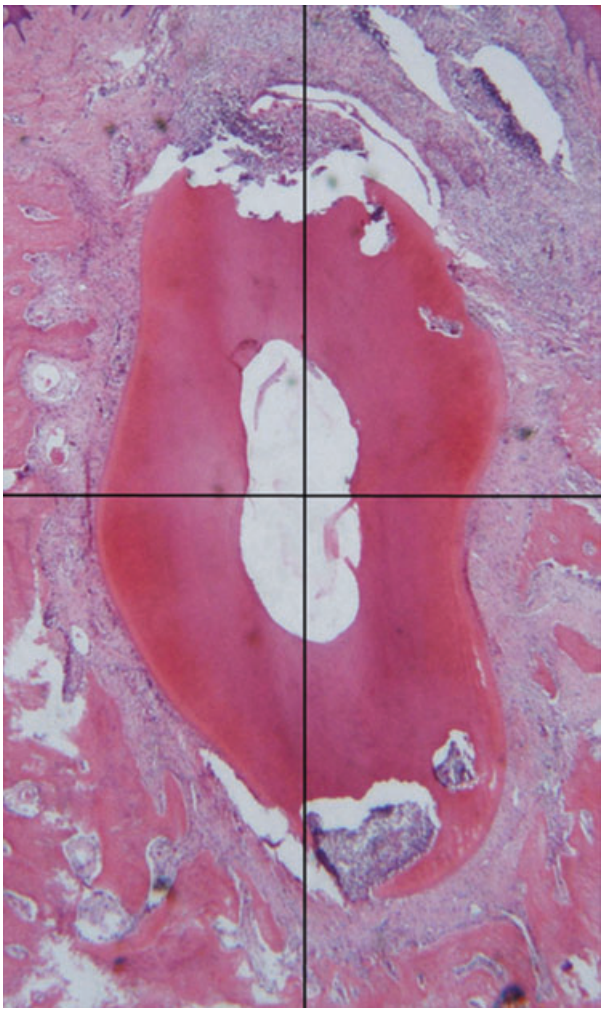

Fig. 4. According to sectioning direction, 0% (mesio-distal) or 100% (facio-oral) will be recorded because of the uneven location resorption in a replanted monkey incisor (affecting almost exclusively 'corner' surfaces).

when presenting the data. Because of the limitations in the size of the samples, it is difficult to prove the precondition of a normal distribution, which is a precondition for using mean and SD and parametric tests (*t*-test, variance tests) and that non-parametric statistics are used to test intergroup differences (16, 59). As mentioned before, it is advisable to consult a statistician before starting an experiment.

Conclusion

In vivo studies in dental traumatology are valuable tools that enable us to understand healing processes after different traumatic injuries. Several critical considerations must be taken into account before embarking on such experimental studies. The authors have tried to share their experience of the most important issues.

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