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Current Status of Animal Experimentation in the Study of Periodontal Diseases and Therapeutics.

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Review Article

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ABSTRACT

Animal models are required to objectively evaluate the physiology and pathogenesis of human periodontal diseases and its various treatment modalities. Selection of the appropriate animal model depends on the similarity of the periodontium and the nature of the disease to that of humans. The more commonly used animal models for studying the pathogenesis of periodontal disease, use of implants and guided tissue regeneration have been dogs and nonhuman primates. Rats and hamsters are best suited for caries and calculus research. Variables unique to each animal species are manifested by a wide range of clinical and histopathological features. Different species have distinct diets, habits, life spans, tissue structures, host defence mechanisms and genetic traits. This article describes the diversity seen in animal models used to study periodontal disease and its prevention and treatment.

INTRODUCTION

Animals are used to understand basic biology, as "models" for studying human biology and disease, and as test subjects for the development and testing of drugs, vaccines, and other biologicals (i.e. antibodies, hormones, ingredients in vaccines, etc.) to improve and advance human health. Animal models have contributed new knowledge in biological sciences, including periodontology [1,2]. Among the animal kingdom, rodents, rabbits, pigs, dogs, and nonhuman primates have been used to model human periodontitis, each with its own advantages and disadvantages [3]. Periodontitis is a highly prevalent, chronic immunoinflammatory disease of the periodontium that results in progressive loss of gingival tissue, the periodontal ligament, and adjacent supporting alveolar bone. Periodontitis has been associated with systemic diseases, such as cardiovascular complications [4], rheumatoid arthritis [5], and adverse pregnancy outcomes [6]. Although cultured cells can be used to study physiological processes that occur during the pathogenesis of periodontitis, the complex host response fundamentally responsible for this disease cannot be reproduced *in vitro*.

Different Animal Models in Periodontology

Non-Human Primates

Monkeys have the advantage of probably being philogenetically similar to humans. All non-human primate species offer a wide range of sizes: from about 300 to 350 gram for certain marmosets, to sizes similar to humans for the largest such as chimpanzees and gorillas. All these species are diphyodont. Macaques, baboons and chimpanzees have the same dental formula as human: I 2/2, C 1/1, Pm 2/2 and M 3/3. The anatomy of teeth and roots is close to that of humans, but the size is smaller. Similar dental microflora, and disease to humans. They show natural or experimentally induced periodontitis [3].

Dogs

Many experimental studies on gingival and periodontal diseases have been conducted in dogs. The beagle is one of the most commonly used due to its size and its extremely cooperative temperament. Globally, all periodontal tissues and the size of the teeth are quite similar to those observed in humans. In dogs, the subgingival

plaque involves predominantly anaerobic gram negative cocci and rods such as, *P. gingivalis* and *F. nucleatum*, similar to human bacteria [7]. However, some major differences exist between dogs and humans such as the lack of lateral movements, no occlusal contacts for all the premolars and presence of open contacts between teeth. The frequent lack of gingival sulci and crevicular fluid, a different composition of periodontal plaque and calculus are other important differences between dogs and humans [8]. All dogs are diphyodont with deciduous and permanent dentition. The formula for permanent dentition is I 3/3, C1/1, Pm 4/4, M 2/3. All domestic dogs have a natural susceptibility to periodontal diseases in adult age but may be maintained healthy by appropriate plaque control. As a limitation of the natural periodontal diseases, the extent and localization of periodontal lesions are not always synchronized in dogs. Periodontal alterations, including gingivitis and periodontitis, increase in prevalence and severity with age, faster than in man but with the same etiologic factors [3].

Rodents

Rodents provide some unique characteristics to evaluate microbial and host responses to complement primate and human periodontal studies.

Rats

Rats are often used in models of experimental periodontal pathogenesis because periodontal anatomy in the molar region shares some similarities with that of humans. Typical rodent dentition is I 1/1, C 0/0, Pm 0/0, M 3/3. The incisor is rootless. The structure of the dental gingival area in rats is quite similar to that observed in humans [10], with a shallow gingival sulcus and attachment of the junctional epithelium to the tooth surface. However, there are some differences: the first is the keratinisation of the crevicular epithelium in rats; the second is the relationship between the gingival and junctional epithelium with desmosomal contact between the most superficial cells of the gingival epithelium and the non keratinized cells of the junctional epithelium [9]. There is clear evidence from the literature demonstrating horizontal bone loss in rats infected with *Aggregatibacter (Actinobacillus) actinomycetemcomitans* or *P. gingivalis*.

Mice

African dormice (*Graphiurus* spp.) are small nocturnal rodents that currently are uncommon in laboratory settings. Their use may increase as they have recently been shown to develop an infection with monkeypox virus and may prove to be a valuable animal model for infectious disease research. Much of the current research involving dormice revolves around field studies and the ongoing taxonomic characterization of the family. As visual speciation of *Graphiurus* is difficult, speciation usually is accomplished by using karyotypic and anatomic variation. African dormice are the only members of the Gliridae family that are located solely in sub Saharan Africa. The other members of the Gliridae family, the Glirinae and Leithiinae, are widely distributed geographically and more commonly used in research [11].

Horses

Common naturally occurring oral diseases in horses include buccal abrasions, calculus, gingival recession, and periodontal pockets. According a recent equine survey, the prevalence of periodontal pockets and gingival recession is highest in older horses and mostly associated with other dental disorders and tooth loss [12]. Because of their size and husbandry considerations, horses are not a practical model for basic science studies of periodontitis or for testing of potential therapies.

Rabbits

Rabbits have mainly been used for testing biomaterials or for treatment of peri-implantitis. However, transcortical drilled holes creating tibial or radial critical-sized femoral defects are traditionally the most commonly used models in rabbits. These defects in long bone are far from the specific situation of periodontal diseases but appear as a very interesting model for testing the bone healing, but they have been found less suitable for regeneration of periodontal ligament [13].

Characterization of the oral microorganisms in rabbits showed numerous pathogenic bacteria, including *F. nucleatum, P. heparinolytica, Prevotella spp., P. micros, S. milleri* group, *A. israelii,* and *A. haemolyticum*, which is somehow consistent with the flora related to periodontal disease in humans [14].

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Advantages and Disadvantages of Animal Experimentation

Advantages of Animal Experimentation

- Biologically similar to and similar diseases as humans- animals suffer from some of the same ailments that humans do. Chimpanzees share more that 99% of DNA with humans and mice share more than 98% DNA with humans, therefore, animals are susceptible to many of the same health problems as humans.
- Relatively short life spans- they can be studied throughout their whole life span or across several generations. Several generations of an animal can be tested for the same health problem as they reproduce at a faster rate. So the effects of the diseases and treatment options can be followed through generations.
- Never run out of organisms – as few animals are bred specially for the purpose of experimentation scientists never run out of number of animals for research.
- Environments can be altered very easily- Most Biomedical experiments need to incorporate one variable at a time to an experiment setting, which would be hard to do with human test subjects.

Disadvantages of Animal Experimentation

- Basic husbandry issues – strict regulations for animal maintenance.
- Ethical issues- the testing of animals for Biomedical Research is much debated.
- Similarity between the results between animals and humans is questioned at times. Profound differences in anatomy, physiology and biochemistry between humans and animals make animals poor models for humans. If a substance produces certain effects at the tissue periphery, these may be masked by metabolic or detoxification mechanisms in nonhuman mammals which are not present in man.
- Critics of animal experimentation, increasingly stress the potential harms that might befall researchers involved in performing such studies. These critics maintain that moral sentiments can be deadened by persistent exposure to animal suffering.
- Animal experiments are expensive.

Animal Studies Done In Periodontal Research

Animal studies have been performed using various animal models to gain the knowledge of anatomy and physiology of the gingiva and periodontium, also to understand the pathologic basis of different diseases and to evaluate different treatment modalities, newer drugs and materials used in the treatment of periodontal diseases.

Experiments done on beagle dogs to study disease progression concluded that disease spreads along the blood vessels and the pathway of spread of the inflammatory cell infiltrate, the anatomic relationships of the teeth to each other and the original morphology of the alveolar bone may influence the pattern of bone loss in periodontitis [15].

A study was done aiming determination of the half-life of periodontal collagen fibres around rat molars. The data indicated that long-lived collagen fibres do not exist in the soft tissues of the periodontium, and are probably not responsible for relapse. The differences in collagen half-life might be caused by local variations in compressive strain induced by normal function [16].

Using the subcutaneous chamber model in mice, a study was carried out to investigate the effect of stress on host response to *Porphyromonas gingivalis.* The results suggest that the levels of TNF-α induced by p. gingivalis in the infection site are down-regulated in stressed animals, and CS is not the sole mediator responsible. The stress-induced reduction in TNF-α level might have an impact on the pathogenesis of periodontal disease in humans experiencing emotional stress [17].

An attempt was made to determine whether the host immune system, and in particular the formation of immune complexes, is involved in the periodontal destruction. In a study, it was seen that the formation of immune complex appears to be involved in the acute phase of periodontal destruction and that the biological activity of antigens is also important [18].

In one study three months after tooth extraction, 72 sandblasted acid etched chemically modified implants were placed in six dogs. Chemically modified, sandblasted acid-etched–surfaced implants with NMC presented crestal bone gain after 3 and 12 months under loading conditions in the canine mandible. The implant design and surface were determinants in the marginal bone level preservation [19].

Studies were done in animals on inflammatory markers [20], outcomes of using - Rh-BMP in dogs [21], PRP in flap strength [22], immunization against periodontitis [23] effects of hormone on bone [24] effects of various drugs on

periodontium [25], implant treatment [26], periodontal regeneration [13]. Studies had given the results which are implicable in humans.

Controversies

In spite of the advancement in biomedical research, and the benefits derived by the society through them, the opposition to animal experiments always existed. Animals in laboratories are routinely subjected to painful procedures. They are burned, shocked, poisoned, isolated, starved, forcibly restrained, addicted to drugs and brain-damaged – and they are usually killed afterwards. No procedure, no matter how painful, redundant or pointless, is prohibited by law. In addition to the physical pain animals endure, many studies have shown marked stress responses in animals undergoing common laboratory procedures. Stress responses in animals are also seen during caging, isolation, handling and blood collection. This not only compromises research results but also graphically illustrates the trauma animals endure in laboratories.

A study of 20 reviews of animal tests' accuracy found that only two concluded that the animal tests were consistent with the human findings or had contributed significantly to developing new treatments.

The animal activists do not appreciate the use of animals in biological studies. They are equating cruelty to animals committed by corrupt leather and meat traders to animal experiments conducted for scientific investigations.

Although cultured cells can be used to study physiological processes that occur during the pathogenesis of periodontitis, the complex host response fundamentally responsible for this disease cannot be reproduced *in vitro*.

Guidelines for Research Publications Involving Animal Experiments

Appropriately designed experiments those minimise variation, provide standardised optimum conditions of animals care and minimise unnecessary stress or pain, often yield better more reliable data.

The guidelines for reporting animal research referred to as ARRIVE (Animals in Research: Reporting In Vivo Experiments) [27], have been developed using the CONSORT (Consolidated Standards of Reporting Trials) [28] Statement as their foundation. These guidelines can be applied to any area of bioscience research using laboratory animals, and the inherent principles apply not only to reporting comparative experiments but also to other study designs. The guidelines provide a checklist that can be used to guide authors preparing manuscripts for publication, and by those involved in peer review for quality assurance, to ensure completeness and transparency.

These guidelines improve reporting of research using animals Guide authors as to the essential information to include in a manuscript, and not be absolutely prescriptive. Be flexible to accommodate reporting a wide range of research areas and experimental protocols. Promote reproducible, transparent, accurate, comprehensive, concise, logically ordered, well written manuscripts. Improve the communication of the research findings to the broader scientific community.

All the countries have set their legislations for conducting experiments on animals. Those rules have to be followed while using animals for experimentation in that country.

Alternatives for Animal Experimentation

In 1959, William Russell and Rex Burch published "The Principles of Humane Experimental Technique". Many of those who oppose animal experimentation would also agree that until animal experimentation is stopped, Russell and Burch's 3Rs provide a means to improve animal welfare. These 3R's refer to replacement, reduction and refinement. They proposed that if animals were to be used in experiments, every effort should be made to Replace them with non-sentient alternatives, to Reduce to a minimum number of animals used, and to Refine experiments which used animals so that they caused the minimum pain and distress and other adverse effects suffered at any time during the life of the animals involved, and enhances their wellbeing. If animals were to be used, as few as possible should be used and they should experience a minimum of pain or distress.

Alternatives to the use of animals in research, testing, and education should be used to lessen the animal number and sufferings. The alternatives may include reduction in the number of animals used, replacement of animals with a non-animal model or with animals of a species lower phylogenetically, or refinement of methods to minimize pain and distress of animals used [27].

Instead of animal, tissue cultures, computerised simulative models can be used to replace the animals in experimentation. Development of alternate strategies to animal experimentation can be part of the objective of the National Centres or a separate Institute for alternate research methodology and validation can be established.

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CONCLUSION

An animal experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available. When an experiment has to be performed, the choice of species shall be carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results should be selected. All experiments shall be designed to avoid distress and unnecessary pain and suffering to the experimental animals. In vitro alternate methods cannot replace animal experimentation totally but can work only as adjuncts and reduce the number of animals to the extent possible. This is why the use of animals continues to be mandatory to meet the statutory requirements. However, efforts to develop alternate methods should continuously be made so that the day will be reached when no more animals are used for experimentation.

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