Primate Model for Testing Periodontal Treatment Procedures: II. Production of Contralaterally Similar Lesions*

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MANY PROCEDURES for treating periodontal pockets are designed to produce new connective tissue attachment to a root surface which had been pathologically exposed and covered by epithelium.[1-4] Some techniques for pocket elimination apparently can cause loss of connective tissue attachment.[5, 6] It is difficult, however, to substantiate such claims unless controlled studies are performed in which comparable areas of connective tissue destruction have occurred on the same tooth surfaces bilaterally. In a histologic investigation of localized periodontal pockets produced in Rhesus monkeys by orthodontic elastics, it was shown that connective tissue attachment was destroyed, and did not regenerate spontaneously after elastic removal.[7] If it were possible to create periodontal pockets with approximately the same dimensions bilaterally in the Rhesus monkey, this primate model would be available for controlled evaluation of periodontal therapy.

The purpose of the present investigation was to: (1) Produce contralateral periodontal lesions with approximately the same dimensions. (2) Develop histometric methods to analyze the significant measurable parameters. (3) Develop a design for future studies of periodontal treatment procedures.

MATERIALS AND METHODS

Four young adult, male Rhesus monkeys (Macaca mulatta), fully conditioned and in good health, were utilized in this study. Orthodontic elastics§ were doubled and placed around the teeth apical to the contact points as described in a previous paper.[7] Elastics were placed bilaterally on maxillary and mandibular central incisors and first molars, and on maxillary first and mandibular second premolars. The elastics were changed at 2-week intervals. The time of elastic placement was staggered because the rate of pocket formation varies on different teeth. From the previous study,[7] the time required for pocket formation on different teeth was known.

Pocket measurements, made with a periodontal probe from fixed points on the tooth surface, were utilized to estimate the depth of the pockets in order to determine when to remove the elastics. The time required to produce pockets, 5 to 8 mm in depth, in this group of monkeys was as follows:

	days
Maxillary centrals	66–84
Maxillary first bicuspids	82–84
Mandibular centrals and second bicuspids	82–84
Mandibular first molars	112–168
Maxillary first molars	167–176

The animals were sacrificed with an intravenous overdose of sodium pentobarbital‖ 12 weeks after removal of the elastics. Immediately after sacrifice, the mandibles and maxillae were dissected free, placed in a fixative¶ for 72 hours, washed for 24 hours in filtered running tap water and placed in 4% nitric acid for decalcification. When decalcification was complete, the jaws were cut into blocks, each containing the experimental and adjacent teeth of the involved area segment. The blocks were washed in filtered running tap water for 24 hours and were processed for celloidin embedding. They were then embedded for mesio-distal sectioning in such a manner that sectioning began from the facial aspect. The blocks were aligned so that the sections were cut parallel to the long axis of the teeth. Serial sections were cut through each block with the microtome set at 12 μ, were stained with hematoxylin and eosin, and mounted.

The cemento-enamel junction (CEJ) was used as a fixed reference point, and the distances between CEJ and various landmarks of the periodontal tissues were measured (Fig. 1). The measurements included: (i) the distance between the CEJ and the apical end of the junctional epithelium (CEJ-JE); (ii) the distance between the CEJ and the crest of the interdental bone (CEJ-CR); (iii) the distance between the CEJ and the apical end of the angular bony defect (CEJ-AAD); and finally, (iv) the distance between the crest of the interdental bone and the apical end of the angular bony defect (CR-AAD). This last measurement was computed using ii and iii above for all pockets which were infrabony in character. A pocket was judged infrabony when the apical end of the junctional epithelium was 1 mm or more apical to the crest of the interdental bone.

Measurements were made with a calibrated grid in the eyepiece of a light microscope at 35 × on the midsection and on step serial sections at 192 μ intervals. Only the

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§ Unitec, Englewood Cliffs, N.J. 1/8 inch medium, intraoral.

‖ W. A. Butler Co., Columbus, Ohio.

¶ Lavdowsky's solution: formaldehyde, 300 ml; glacial acetic acid, 105 ml; 95% ethyl alcohol, 1500 ml; distilled water, 1200 ml.

interproximal pocket areas were analyzed. The anatomy of the maxillary first bicuspid was such that a pocket could be created only on the distal surface and, therefore, the mesial surface of this tooth was not included in the analysis.

Thirty-one pockets from the right side of the animals were compared with the corresponding pockets on the contralateral side by the paired-sample t test.[8] If the measurements on the right side are denoted by x and those on the left by y, the paired differences, $d_i = y_i - x_i$ for $i = 1, 2 \ldots, 31$ are averaged. The test statistic $t = \sqrt{31}\,\bar{d}/\mathrm{sd}$, where d denotes the mean and sd the standard deviation of these paired differences, may be compared with the t distribution with 30° of freedom in order to test the hypothesis that the differences have a mean of zero, i.e., that there is no difference between the left and right sides. Regardless of the results of the t test, a correlation between the left side and right side paired measurements is also required in order to demonstrate that knowledge of the dimensions on one side offers sufficient information to make an accurate prediction of the dimensions on the contralateral side. Accordingly, Pearson's product-moment correlations were run on the 31 pocket pairs for each of the three parameters. In addition, the data were analyzed separately for each monkey, for all combinations of two monkeys, for suprabony and infrabony pockets and for shallow (<3 mm) and deep (>3 mm) pockets. All statistical analyses were accomplished using MIDAS (Michigan Interactive Data Analysis System) developed by the Statistical Research Laboratory at the University of Michigan.

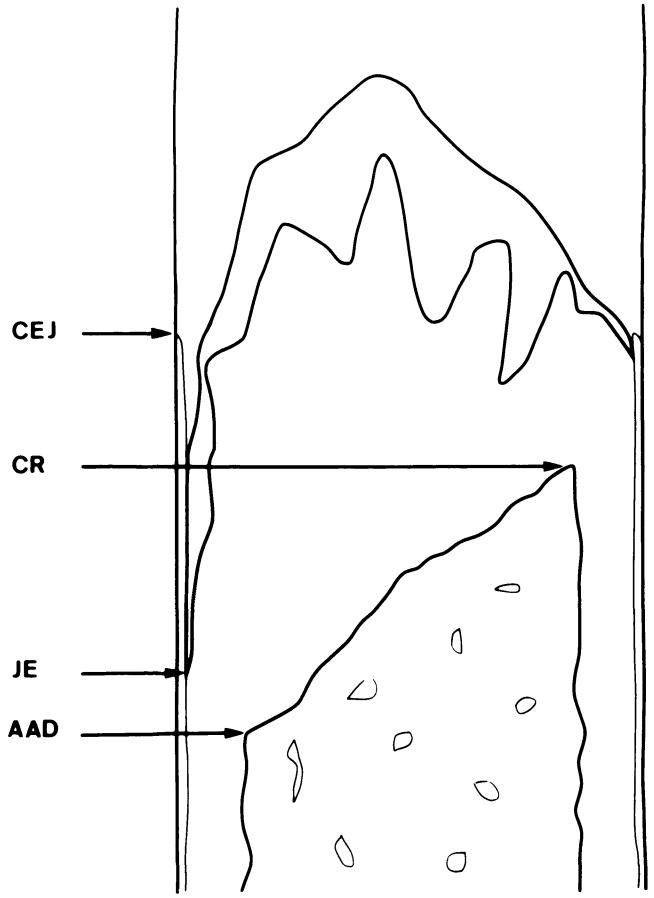

FIGURE 1. *Diagram representing the interproximal periodontal tissues seen in a mesio-distal section. Using the cemento-enamel junction* (CEJ) *as a fixed reference point, a linear measurement can be made along the root surface of the level of the connective tissue attachment* (JE), *crestal bone* (CR) *and the apical end of the angular bony defect* (AAD).

RESULTS

The results of the paired difference t tests are summarized in Tables 1–6. Both the midsection and step serial section results are given in successive tables for each of the four parameters in each monkey.

Tables 1 and 2 give the data comparing the contralateral loss of connective tissue attachment. When step serial sections were used there were no significant differences between the right and left side for any monkey individually or when all four monkeys were taken as a whole. In addition, the mean of the differences was very small, ranging from 0.01 mm to 0.10 mm. When only the midsection measurement was utilized, monkey no. 4 showed what may be significant differences between the right and left sides, the mean of the differences being 0.40 mm with a P value of 0.05. The remaining monkeys demonstrated no significant differences between sides.

Tables 3 and 4 present data comparing contralateral loss of crestal bone height. While the data were more variable than those for loss of attachment measurements, there were again no significant differences

TABLE 1. *Distance between Cemento-Enamel Junction and Apical End of Junctional Epithelium (Midsection Measurements)*

Monkey	Number of paired pockets	Mean		Mean of Differences ± S.D.	T Value	P
		Left	Right			
		mm				
1	8	2.71	2.63	0.08 ± 0.25	0.88	0.41
2	9	3.08	3.08	0.00 ± 0.50	0.00	1.00
3	9	1.84	1.89	−0.05 ± 0.28	−0.55	0.59
4	5	2.14	2.54	−0.40 ± 0.32	−2.80	0.05
Total	31	2.47	2.53	−0.06 ± 0.34	−0.99	0.32

TABLE 2. *Distance between Cemento-Enamel Junction and Apical End of Junctional Epithelium (Mean of Step Serial Sections, 192 μ)*

Monkey	Number of paired pockets	Mean		Mean of Differences ± S.D.	T Value	*P*
		Left	Right			
		mm				
1	8	2.47	2.40	0.07 ± 0.14	1.43	0.20
2	9	2.91	3.01	−0.10 ± 0.37	−0.81	0.44
3	9	1.73	1.74	−0.01 ± 0.20	−0.27	0.80
4	5	2.23	2.31	−0.05 ± 0.29	−0.69	0.53
Total	31	2.34	2.37	−0.03 ± 0.25	−0.67	0.54

TABLE 3. *Distance between Cemento-Enamel Junction and Crest of Interdental Bone (Midsection Measurements)*

Monkey	Number of paired pockets	Mean		Mean of Differences ± S.D.	T Value	*P*
		Left	Right			
		mm				
1	8	2.57	2.58	−0.01 ± 0.19	−0.28	0.79
2	9	3.20	3.03	0.17 ± 0.34	1.08	0.31
3	9	1.98	2.02	−0.04 ± 0.31	−0.34	0.75
4	5	2.60	2.85	−0.24 ± 0.30	−1.84	0.14
Total	31	2.57	2.59	−0.02 ± 0.28	−0.40	0.69

TABLE 4. *Distance between Cemento-Enamel Junction and Crest of the Interdental Bone (Mean of Step Serial Sections, 192 μ)*

Monkey	Number of paired pockets	Mean		Mean of Differences ± S.D.	T Value	*P*
		Left	Right			
		mm				
1	8	2.31	2.42	−0.11 ± 0.13	−2.29	0.06
2	9	2.94	3.01	−0.07 ± 0.29	−0.76	0.47
3	9	1.91	1.87	0.07 ± 0.23	0.49	0.64
4	5	2.59	2.61	−0.02 ± 0.23	−0.20	0.85
Total	31	2.42	2.46	−0.04 ± 0.22	−1.02	0.32

between right and left sides. In the measurements CEJ-AAD (Tables 5 and 6) there was a significant difference between contralateral teeth in monkey no. 1 when measured with step serial sections even though the mean of the differences was only 0.11 mm.

Seventeen paired pockets were infrabony in nature. On these, the depths of the angular bony defects on contralateral surfaces were compared and found to be very similar. The *P* values for midsection measurements and step serial section measurements were 0.91 and 0.60 respectively.

When suprabony and infrabony pockets were analyzed separately and when shallow (<3 mm) and deep (>3 mm) pockets were subjected to separate analysis, there were no significant right-left differences.

Similarly, when any two monkeys were analyzed together there were no significant differences in the four parameters measured.

The correlation coefficients for CEJ-JE, CEJ-CR and CEJ-AAD were 0.95, 0.96 and 0.94, respectively.

DISCUSSION

The results of this study demonstrated that experimental defects can be produced with elastics on contralateral tooth surfaces with a small degree of variability in the important parameters. Thus, this experimental model could appropriately be used to test the effects of periodontal therapy. More specifically, the magnitude of the paired differences was shown to be insignificant utilizing the paired sample t-test when step serial sections were utilized. The right side, left side correlation indicated nearly perfect correspondence of pocket depth

TABLE 5. *Distance between Cemento-Enamel Junction and the Apical End of Angular Defect (Midsection Measurements)*

Monkey	Number of paired pockets	Mean		Mean of Differences ± S.D.	T Value	*P*
		Left	Right			
		mm				
1	8	3.12	3.16	−0.04 ± 0.39	−0.28	0.79
2	9	3.48	3.53	−0.05 ± 0.50	−0.31	0.77
3	9	2.53	2.46	0.07 ± 0.45	0.46	0.66
4	5	2.85	2.60	0.25 ± 0.30	1.84	0.14
Total	31	3.01	2.97	0.04 ± 0.42	0.53	0.61

TABLE 6. *Distance between Cemento-Enamel Junction and Apical End of Angular Defect (Mean of Step Serial Sections, 192 μ)*

Monkey	Number of paired pockets	Mean		Mean of Differences ± S.D.	T Value	*P*
		Left	Right			
		mm				
1	8	2.79	2.89	−0.11 ± 0.10	−3.01	0.02
2	9	3.41	3.43	−0.02 ± 0.45	−0.15	0.88
3	9	2.41	2.36	0.05 ± 0.21	0.68	0.51
4	5	2.77	2.72	0.04 ± 0.19	0.49	0.65
Total	31	2.86	2.87	−0.01 ± 0.26	−0.22	0.84

measurements as long as contralateral pairs were selected. This method implies a model which will be used in the future to test procedures, and, as such, the stability of the correlations is critical. The 99.7 percent confidence intervals on the lower boundary of the correlation coefficients are 0.90, 0.91 and 0.87 for the measured parameters. This indicates that in future studies utilizing the model, we can depend on obtaining a high enough degree of correspondence between preoperative paired pocket dimensions to be able to detect postoperative right-left differences.

The results of this study also provided some guidance on the design of future investigations of this type. For example, a study with this model system could be designed to test the probability and extent of reattachment. According to Chilton,[8] the number of paired surfaces, N, required to ensure a 95% probability that an average difference in attachment of 0.30 mm would be significant at the 1% level of significance can be obtained as the solution of the equation

$$2.33\left(\frac{\text{sd}}{\sqrt{N}}\right) + 1.64\left(\frac{\text{sd}}{\sqrt{N}}\right) = 0.30 \text{ mm}.$$

Since our results indicate that the standard deviation is approximately 0.30 mm (c.f. Tables 1–6) this gives $\sqrt{N}$ of almost 4, i.e., $N \geqq 16$ would satisfy the stated criteria for the experimental design. Since the use of two monkeys would result in 18 to 22 pairs of surfaces to compare, the adaption of our experimental model utilizing two monkeys would result in a sensitive comparison (95% power) of treatment effects in which treatment differences of as little as 0.30 mm would be significant at the 1% level of significance. While changes in the level of significance, the required power of the test and/or the use of a more conservative estimate of sd, affect the value of N as computed above, the use of two monkeys satisfies a wide range of experimental requirements indicating that the experimental model described in this paper may be used to provide accurate estimates of treatment effects at minimal cost.

The utilization of an animal model to test and develop periodontal treatment procedures allows precise histometric evaluation and precise controls. Because this is a primate model, the anatomy and physiology of the periodontal tissues are quite similar to those of man. The limitations of such a model, however, must be clearly understood. Such a system should be used to test new treatment procedures (and to answer questions about existing procedures) before they are used in man. If they show promise in the model and are free from harmful effects then controlled clinical trials in man would be indicated.

Studies are currently underway with this model to define the effects of various types of periodontal therapy.

SUMMARY

Experimental periodontal pockets were produced bilaterally in four Rhesus monkeys. The pockets were

analyzed histometrically and the resultant data were subjected to statistical analysis. There were but few significant differences between pockets on contralateral tooth surfaces, and in each case, the magnitudes of the observed differences were so small as to be of no biological significance. The use of this model system for future studies on periodontal treatment was discussed. It was shown that by utilizing only two monkeys, the model system can discern treatment effects on the measurable parameters of as little as 0.3 mm.

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Dr. William J. Takacs
1929–1976

Colonel William J. Takacs, the Air Force representative to District 8 of the American Academy of Periodontology, died January 2, 1976.

A 1951 graduate of Case Western Reserve School of Dentistry, he completed his periodontal residency through the combined University of Texas/Wilford Hall USAF Medical Center program in 1964.

Dr. Takacs had been Chairman of the Department of Periodontics at Wilford Hall USAF Medical Center, Lackland AFB until retiring in December due to ill health.

His career in periodontics was highlighted by being director and teacher in all three of the Air Force's Advanced Periodontic Residencies at Lackland, Andrews and Wright-Patterson Air Force Bases. He received the Air Force Commendation Medal for his work in developing the residency program at Wright-Patterson AFB and the Meritorious Service Medal for his efforts at the Malcolm Grow USAF Medical Center at Andrews. He was further honored by being selected as senior consultant to the Air Force Surgeon General in periodontics and Special Consultant in Periodontics to the Assistant Surgeon General for Dental Services.

Bill was a Diplomate of the American Board of Periodontology and a Fellow of the American College of Dentists.

The Air Force, District 8 and periodontics has lost a great leader, teacher, clinician and friend.

He is survived by his wife, Irene, and sons Mark, Tim and Phillip of San Antonio, his daughter Susan Krueger of Laurel, Md., his mother, Elizabeth Takacs of Dearborn, Michigan and brother, John, of Anaheim, California.