



BIOTECHNICS

F O C U S E D E X P E R T I S E

BriteSmile Whitening System

FINAL REPORT

PULP EVALUATION

AUTHOR - Milton V. Marshall, Ph.D., D.A.B.T.

STUDY COMPLETION DATE: May 9, 2002

CONDUCTED BY:
Biotechnics, Inc.
310 Millstone Drive
Hillsborough, NC 27278

LABORATORY STUDY NUMBER:

24-02

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SUBMITTED TO:
BriteSmile, Inc.
490 N. Wiget Lane
Walnut Creek, CA 94598


CONFIDENTIAL

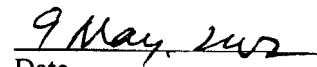
STUDY INFORMATION

Test Article Action: Tooth Whitener
Test Article Identity: BriteSmile Tooth Whitening System
Lot No. 1221
Expiration date: January 31, 2004
Description: Clear gel
Stability: Stable for the duration of the study
Storage Conditions: Refrigerate (2-8°C)
Handling Precautions: Routine Laboratory
Supplier: BriteSmile, Inc.
Barrier material: lot #A27108, exp July 31, 2003
Masking cream: lot #1104, exp September 30, 2003
Preparation of Test Article: as supplied
Test System Identity: Cynomolgus monkeys
Diet: Harlan Teklad Certified Primate Diet
#2055
Chemicals and Supplies:
2.00N H₂SO₄ Ricca Chemical Co. Lot #2102285, exp May 30, 2002
0.100N KMnO₄ Ricca Chemical Co. Lot #2107030, exp December 31, 2002
H₂O₂ Mallinkrodt AR (ACS) 31.3% Lot#5240 745A05, exp November 30, 2002
Formic acid, 94.1%, ACS J.T. Baker Co. Lot#V15476, exp December 31, 2004
Water – Biotechnics, Inc. deionized, Analyzed July 30, 2001
Buret Kimble 17027F-10 (T.D.) Serial #8098, certified May 31, 2001
Study Initiation Date: April 9, 2002
Study Completion Date: May 9, 2002
Test Facility (In-life study): LAB Pre-Clinical Research International Inc.
560 Cartier Blvd West, Laval, Québec,
Canada
Study Personnel (In-life): A.C. Zerouala, D.V.M., Ph.D.
Jean-Paul Descôteaux, D.V.M., Ph.D.
Shelley Watson, B.Sc., M.Sc.
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George A. Parker, DVM, PhD, DACVP, DABT
Mark Forsell, AB, HT(ASCP)
Lynn Cates, HT
Test Facility (Histopathology):
Study Personnel (Histopathology):

COMPLIANCE STATEMENT

This study was conducted in accordance with the standard operating procedures of LAB Pre-clinical Research International, Inc. and Biotechnics, Inc. No deviations in the study protocol or standard operating procedures occurred to adversely affect the integrity of the data in this study.


Milton V. Marshall, PhD, DABT
Study Director


Date

SUMMARY

The purpose of this study was to evaluate the effect of the BriteSmile Whitening System on tooth pulp following topical application in *Cynomolgus* monkeys. Four females were treated topically for three 20-minute periods with the BriteSmile tooth whitening system that included light activation. Animals were sedated prior to application with pentothal, and the test article was applied to a minimum of two upper and two lower teeth; contralateral teeth were untreated and used as controls. The BriteSmile tooth whitening system gel was applied to teeth on one side of the mouth (upper and lower arches). A visible wavelength gas plasma light source (400-505 nm) was placed approximately 6 cm from the treated teeth, and light was directed onto the teeth for a period of 20 minutes. This procedure was repeated twice for a total exposure time of 60 minutes; fresh whitening gel was applied prior to each 20-min treatment. Following treatment, 4 upper (2 treated, 2 untreated) and 2 lower (1 treated, 1 untreated) teeth were extracted at 20 and 44 hours, respectively. Extractions were performed under isoflurane anesthesia. The roots of all teeth were broken, and the teeth were placed in 10% neutral buffered formalin for 3-4 days. After fixation, teeth were decalcified in 10% formic acid. Following processing, slides were prepared from multiple sections of each tooth. Slides were stained with hematoxylin and eosin (H&E) or Masson's trichrome stain. All slides were evaluated for treatment-related alterations in a blind manner. No treatment-related alterations in teeth were observed following exposure to the BriteSmile tooth whitening system.

MATERIALS AND METHODS

Test System

Monkeys were selected to satisfy regulatory requirements for safety testing in a non-rodent species because the dentition of primates is more closely related to that of humans than other species. The *Cynomolgus* monkeys assigned to this study were obtained from Covance Research Products Inc., P.O. Box 540, Alice, TX 78333 and Government of Canada, Health and Welfare Canada, Animal Research Division, Tunney's Pasture, Ottawa, Ontario K1A 0L2. At the start of treatment, the animals were 3-4 years old and had a body weight range of 3.7 to 5.0 kg.

Animal Management

Upon transfer from LAB Pre-Clinical Research International Inc.'s spare colony, a detailed physical examination and body weights were performed on all animals under the direction of the clinical veterinarian. Animals were uniquely identified by the supplier by means of a permanent tattoo on the chest. Following animal assignment, cages were clearly labeled with a color-coded cage card indicating study number, animal number, sex, and treatment. The monkeys were housed individually in stainless steel cages equipped with an automatic watering system. The animal room environment was controlled as follows: temperature $21\pm 3^{\circ}$ C, humidity 30–70%, 12 hours light/12 hours dark, 12-20 air changes per hour. Temperature and relative humidity were monitored continuously and recorded twice daily.

Diet/Water

A standard certified commercial primate chow was provided to the animals twice daily except overnight prior to tooth extraction. Soft food such as bananas was provided to the animals after tooth extraction. Concentrations of contaminants in the diet (e.g., heavy metals, aflatoxin, organophosphates, chlorinated hydrocarbons and PCBs) are controlled and routinely measured by the manufacturers (Batch certificate on file at LAB Pre-Clinical Research International Inc.).

Municipal tap water (purified by reverse osmosis and treated by ultraviolet light) was provided to the animals *ad libitum* except at least 1 hour prior to tooth extraction. Periodic analyses of municipal tap water and the water which has been purified by reverse osmosis and treated by ultraviolet light are performed by Bodycote Technitrol Inc., Pointe Claire, Quebec. The analytical results are retained on file at LAB Pre-Clinical Research International Inc. There were no known contaminants in the diet and water that would interfere with the assessment of the objectives of the study.

Application of Test Article

Animals were sedated prior to treatment (pentothal, 0.8 ml/kg), which was performed by Dr. Salim Nathoo. Barrier material was initially applied to the gums for protection and hardened by applying a standard dental curing light for approximately 5 seconds. Next, masking cream was applied around the peri-buccal region to protect any exposed tissue. Finally, the test article (hydrogen peroxide-containing whitening gel) was applied topically three times. Following each application, a visible wavelength light source was directed onto the treated teeth for a period of 20 minutes. At the end of each cycle of treatment, the previous test article was removed, and a new layer was applied. The mouths of all animals were rinsed with reverse osmosis water on completion of the procedure to remove any residual test article. The right sides of the mouths were treated in animals 1501A and 1504A; the left sides of the mouths were treated in animals 1502A and 1503A.

Analysis of Test Article

Hydrogen peroxide content was determined by titration with KMnO_4 according to procedures published in the USP. Briefly, a 20-ml volume of 2.0 N H_2SO_4 was added to a 20-ml sample, and this mixture was titrated with 0.1N KMnO_4 until a slight pink color remained in the solution after addition of KMnO_4 . Each ml of 0.1N KMnO_4 is equivalent to 1.7005 mg H_2O_2 , and the following formula was used to determine peroxide content:

$$\% \text{H}_2\text{O}_2 = \frac{(\text{ml KMnO}_4 \text{ titrant}) \times 0.1\text{N} \times 1.7005 \text{ mg H}_2\text{O}_2}{\text{Sample weight (g)}}$$

Three samples of test substance BriteSmile Procedure Gel Lot #1221 were weighed, and the peroxide content was determined as indicated above. A sample of aqueous hydrogen peroxide was also analyzed in duplicate for comparison. Results of the titrations are shown in the table below. All samples were analyzed 13 April, 2002.

Sample ID	Weight (g)	Volume KMnO_4 (ml)	% H_2O_2
BriteSmile-1	0.1042	9.70	15.83
BriteSmile-2	0.0945	8.75	15.745
BriteSmile-3	0.1138	10.55	15.765
Mean \pm SEM			15.78 \pm 0.026
H_2O_2 -1	0.0272	5.20	32.51
H_2O_2 -2	0.0270	5.15	32.44
Mean \pm SEM			32.48 \pm 0.035

Clinical Observations

Upon transfer, a detailed clinical examination was performed on each animal; cage-side observations of clinical signs were performed daily. On the day of treatment, detailed clinical examinations were performed prior to dosing, and clinical signs (ill health, behavioral changes etc.) were evaluated once a day. Body weights were recorded upon transfer, prior to treatment on Day 1 and on Day 2.

Tooth Extraction

At 20.0-20.4 and 44.2-44.4 hours following treatment 4 upper (2 treated and 2 untreated) and 2 lower (1 treated and 1 untreated) premolars, respectively, were extracted by the Sponsor from each animal under general anesthesia: Acepromazine and Ketamine (0.1mL/kg), followed by isoflurane. An analgesic (buprenorphine, 0.3 mL/kg) was administered to control pain following extraction and 9-12 hours thereafter. Prophylactic antibiotic treatment (Pendure, 0.5 or 1.0 mL) was given 22.8-24.5 hours prior to the first tooth extraction and 45.0-46.4 hours thereafter. Teeth were placed in 10% formalin and shipped to Biotechnics, Inc., 310 Millstone Drive, Hillsborough, NC 27278 on April 12, 2002 for histological examination.

Terminal Procedures

All animals were released to the LAB Pre-Clinical Research International Inc. spare colony following cessation of antibiotic treatment following tooth extraction.

Tooth Decalcification, Processing, Staining, and Evaluation

Teeth were decalcified in 10% formic acid/water for nine days and rinsed for 4-5 hours with water. Rinsed teeth were placed in an automatic tissue processor for paraffin embedding. Sections were removed from paraffin and placed on glass slides. Slides were stained with hematoxylin and eosin or Masson's trichrome stains, and coverslips were affixed to the slides. Pulp chambers of the teeth were evaluated without knowledge of treatment groups. There was no statistical analysis performed on the data other than means and standard deviations because of the small sample size.

Data Retention

All data generated at LAB Pre-Clinical Research International Inc., together with the original protocol, protocol amendment, and summary report will be retained for approximately 10 years in the LAB Pre-Clinical Research International Inc. archives (560 Cartier Boulevard West, Laval, QC H7V 1J1, Canada). LAB Pre-Clinical Research International Inc., agrees to give the Sponsor sufficient advance notification of any intended disposal of such materials after the 10-year holding period to allow the Sponsor to secure alternative storage facilities. Data generated at Biotechnics, Inc. and a copy of the final report will be retained by Biotechnics, Inc. at 310 Millstone Drive, Hillsborough, NC.

Standard Operating Procedures

The Standard Operating Procedures used during the course of this study by LAB Pre-Clinical Research International Inc. and Biotechnics, Inc. are maintained on-file at the respective institutions.

RESULTS AND DISCUSSION

Mortality and Clinical Signs

There was no mortality following the topical application of the test article, and no treatment related clinical signs were observed following topical application of the test article to premolars (Table 1). However, minor and anesthesia related clinical signs of large amount of emesis and moderate amount of white froth and wet, beige material on the cage tray were noted for one female (1501A) approximately 3h after the third application of test article. There was no adverse effect on body weight following topical application of the test article (Table 2).

Tooth Extractions

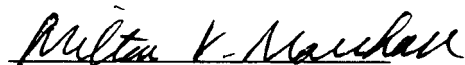
Tooth extractions 4 upper premolars (2 treated, 2 untreated) and 2 lower premolars (1 treated, 1 untreated) were performed 20.0-20.4 and 44.2-44.4 hours, respectively, following treatment. In the numbering system used for human teeth used in the United States, Tooth 1 was anterior and it would correspond to teeth 5 or 12, and tooth 2 was posterior, corresponding to teeth 4 and 13 in this numbering system. The lower teeth removed corresponded to human teeth 20 or 21 and 28 or 29.

Histopathologic Evaluation

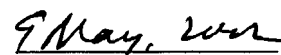
The dentine of teeth from all animals (treated and untreated) was within normal limits one day and two days after treatment. In addition, there was no evidence of treatment-related cellular damage to the pulp (Table 3). Extracellular vacuoles were observed in the pulps of treated and untreated teeth at the same incidence, and they are likely processing artifacts. Epithelial hypoplasia was observed in untreated teeth from two animals two days after bleaching.

CONCLUSION

No adverse reactions were observed in premolars treated with the BriteSmile tooth whitening system one day or two days post-treatment.



Milton V. Marshall, PhD, DABT
Study Director


Date