AAO Foundation Final Report Form

Type of Award, Research Aid Award

Name of Principal Investigator:
Andrea Lou Feather D.D.S., M.S.

Institutions: Western University of Health Sciences College of Dentistry and University of Arkansas for Medical Sciences and Arkansas Children's Hospital.

Title of Project: Does a Popular Acne Medication, Accutane Affect Tooth Movement in Rats?

Period of AAOF Support: 07-01-12 to 6-30-2013

Amount of Funding: $5,000.00

Summary:
This study was a drug test, on the effects of a single dose of a popular acne medication, Accutane, on palatal suture expansion and dental translation and tipping. Accutane is a powerful drug known to alter bone biology. This project involved two experiments. First was an initial pilot study where our sample size was reduced from 20 to 9 animals from a failure of the springs to bond to the maxillary dentition. A poster presentation of the research was presented at the AADR meeting. A manuscript was submitted to *Orthodontics and Craniofacial Research* but was not accepted for publication. Attached is a copy of the manuscript.

Pilot Experiment Abstract:

The acne medication, isotretinoin affects palatal expansion and relapse in a rat model.

Abstract: Young people exposed to high doses of 13-cis-retinoic acid (isotretinoin) are at risk for premature epiphyseal closure, while adults on long-term Isotretinoin therapy are at risk for development of hyperstosis and other abnormal bone changes. Acne preparations containing isotretinoin are commonly used by teenagers who are also receiving orthodontic therapy. Objectives: This investigation was designed to determine if isotretinoin would affect expansion or relapse during palatal expansion therapy in a rat model. Methods: Age and sex matched female (n=5) ~200g Sprague-Dawley rats were treated for 47 days with 2mg/kg/day of isotretenoin IP (a dosage consistent with treatment of recalcitrant cystic acne in humans), or with DMSO vehicle as a control. Impressions of the maxilla were taken before spring placement. The palate was expanded approximately 1mm using stainless steel W-archwire springs bonded bilaterally to the molars delivering 10gm force. The expansion was held for 4 weeks. Impressions were taken at debonding, and after 3 days and 7 days. The intermolar distance for each impression was digitally measured from photographic images of the impressions. Results: Analysis using a 2 sample t-test showed increased expansion of the palate between treated and untreated animals at debonding (1.20 vs 0.96mm respectively, $p<0.03$), and greater relapse at 3 day (0.46 vs 0.31mm, $p<0.08$) and 7 day (0.44 vs 0.27mm, $p< 0.04$) relapse periods.
Conclusions: This study suggests that isotretinoin does not hinder and may increase orthodontic expansion, however, it may also increase the likelihood of relapse after palatal expansion.

Improved Experiment:

The second experiment was done using a stronger bonding material and improved bonding techniques and 20 total data sets were obtained. Two additional animals from the treated and

non-treated groups were used as well for histology but this aspect of the project was not completed as the histologic results were not readable. Additionally, sagittal and coronal sections taken from cone beam computer tomography scans were done as an improvement over using photographs of impressions taken at T1, T2, and T3 to make measurements. This allowed for greater visualization of the internal structures and analysis of cross sections and greater accuracy in measurements. Results of the second experiment was presented at the 2018 AADR Meeting as a Poster Presentation.

Abstract of Improved Experiment:

Acne Medication, Isotretenoin, Affects Tooth Movement in Rats

Abstract: A side effect of *Cis*-Isoretinoic acid, a drug used for resistant cystic acne, is osteopetrosis that can last years. In this animal study, its effect on palatal expansion and tipping and translation tooth movements was investigated.

Twenty-four juvenile Sprague-Dawley female rats were divided into treatment or control groups. The drug was administered before expansion to therapeutic levels and continued. CBCT scans were taken at spring placement (T1) to expand the maxilla laterally. At 3 weeks appliance removal CBCT scans were taken (T2). Scans were repeated after 4 days of relapse (T3). Resulting coronal sections were analyzed for 8 linear and angular parameters using the Anton-Bass analysis in Dolphin Imaging software between T2 and T1 (expansion) and T3 and T2 (relapse). There was significant hindrance to dental tipping of upper and lower molars while maxillary width and upper interdental widths were unaffected. Horizontal palatal width and nasal width were reduced but did not achieve statistical significance. Since dental tipping and sutural expansion was hindered, expansion occurred through bodily buccal tooth movement. This resulted in increased incidence of buccal crossbite and dehiscence. Relapse of molar tipping was significantly less in the experimental group in both arches at 4 days. The upper relapse was 51% in the control group and half that in treated animals. This animal study suggests that orthodontists should query patients on Accutane use and watch for gum recession, expect increased treatment times, and disturbances in palatal expansion. It also suggests that the mechanism of action is broad-based but targeted at periodontally-mediated dental tipping compared to translation or sutural growth or sutural expansion. This study also presents a successful animal model for testing other drug effects on three types of orthodontic movements, dental tipping mediated by the periodontal ligament, sutural expansion and translation of teeth through the alveolus.

In summary, the original aims of the proposal were examined, and the findings suggest that a single therapeutic dose of this powerful acne medication can slow orthodontic tooth movement, will not prevent palatal expansion, can result in less relapse and lastly may result in untoward orthodontic results such as dehiscence. Secondarily we were successful in devising an animal model for testing other drug effects on tooth movement and palatal expansion. Other studies have used animal models with larger extraoral springs, requiring surgical attachment. Our study was done with intraoral springs bonded to the teeth instead.

The results of the second experiment are being prepared for publication in *Orthodontics and Craniofacial Research.* The AAOF support was acknowledged in all. I would like to use AAOF funding for my ongoing and future research efforts.

Arkansas Children's

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Poster #1267

Acne Medication, Isotretenoin, Affects Tooth Movement in Rats

Andrea L. Feather D.D.S., M.S., Bruno Azevedo D.D.S., Alexander Lee D.D.S., Charles Stoianovici D.D.S., Nicolas Bumacod D.D.S., Carlos Guerra, and James L. Borke, PhD Western University of Health Sciences College of Dental Medicine

2018 AADR/CADR Annual Meeting & Exhibition
Session Title: Craniofacial Biology: Bone, Facial Growth and Tooth Movement Abstract #2844232

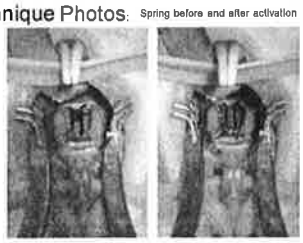
BACKGROUND

Therapeutic doses of Accutane or *Isotretenoin* is known to cause early closure of the epiphyseal plates during growth. Some patients undergoing orthodontic treatment are prescribed this powerful acne medication, for resistant cystic acne. This osteopetrosis can last years. In this animal study, its effect on palatal expansion and tipping tooth movements was investigated.

HYPOTHESIS

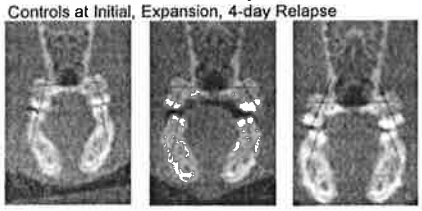
Accutane does not affect sutural expansion, or dental tipping movements in rats when administered as a single course of treatment to juvenile rats at therapeutic levels.

Technique Photos: Spring before and after activation

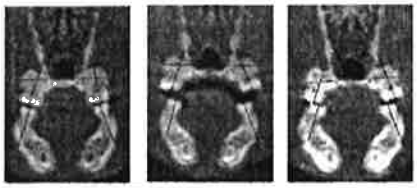


Custom made jig for CBCT Imaging

CBCT Modified Anton-Bass Analysis :
Controls at Initial, Expansion, 4-day Relapse



Experimentals:



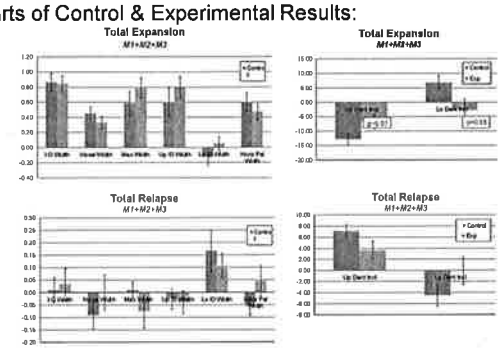
MATERIALS AND METHODS

Twenty-four juvenile Sprague-Dawley female rats were divided into treatment or control groups. The drug was administered before expansion to therapeutic levels of 2mg/kg/day of Isotretenoin IP (a dosage consistent with treatment of recalcitrant cystic acne in humans), or with DMSO vehicle as a control. W-arch springs were passively bonded to the palatal surfaces of the upper molars using BankLok by Reliance Ortho and activated for 1 mm buccal expansion evenly across 6 molar teeth delivering 10 gm force/tooth.

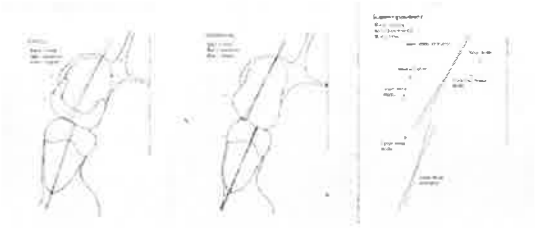
CBCT scans were taken at spring placement (T1). Scans were repeated 3 weeks later at appliance removal (T2) and after 4 days of relapse (T3). Mid-molar coronal sections (3) were analyzed for 8 linear and angular parameters using the Anton-Bass analysis in Dolphin Imaging software between T2 and T1 (expansion) and T3 and T2 (relapse).

Statistical significance was determined using the ANOVA Single Factor analysis with p = .07. Visual inspection of all sections was done for crossbites and dehiscence.

Charts of Control & Experimental Results:



Composite Drawings Control & Experimental and Comparisons:



This research was funded by the American Association of Orthodontist Foundation.

RESULTS

Isotretenoin caused statistically significant hindrance to dental tipping of upper molars (p = .07) and lower molars (p=.05) while maxillary width and upper interdental widths were not statistically different. Horizontal palatal width and nasal width were reduced, but did not achieve statistical significance. Since dental tipping was hindered while palatal width was not, expansion most likely occurred through bodily buccal tooth movement. There was an observed increased incidence of buccal crossbites (4) and dehiscence in the experimental group compared to the controls (1). Relapse of molar tipping was significantly less in the experimental group in both arches at 4 days. The upper relapse was 51% in the control group and half that in treated animals.

CONCLUSIONS

This animal study suggests that a course of Accutane use will not prevent palatal expansion but hinders dental tipping. Additionally, relapse was significantly less in treated animals compared to controls. This study shows that the mechanism of action is broad-based and targeted at periodontally-mediated dental tipping rather than sutural growth or sutural expansion. It suggests that bodily translation occurs through the alveolus. This study presents a successful animal model for testing drug effects on three types of orthodontic movements, dental tipping, sutural expansion and translation of teeth through the alveolus.

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Ertugrul DT, Karadag AS, Tutal E et-al. Therapeutic hotline. Does isotretinoin have effect on vitamin D physiology and bone metabolism in acne patients? Dermatol Ther 2011:24(2):281-5.doi:10.1111/j.1529-8019.2011.01406.x

Luthi F, Eggel Y Theumann N. Premature epiphyseal closure in an adolescent treated by retinoids for acne: an unusual cause of anterior knee pain. Joint Bone Spine. 2012:79(3):314-6.doi:10.1016/j.jbspin.2011.11.001

The acne medication, isotretinoin affects palatal expansion and relapse in a rat model

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Manuscript ID:	Draft
Manuscript Type:	Original Article
Research Area:	Animal experiment, Craniofacial biology, Tooth movement
Keywords (Please write 3 to 5 keywords according to Index Medicus):	Accutane, isotretinoin, palatal expansion, retinoic acid, rat model

The acne medication, isotretinoin affects palatal expansion and relapse in a rat model

C.C. Gage[1]
A. Feather[1]
C. Stoianovici[1]
C. Guerra[1]
J.L. Borke[1]

[1]Western University of Health Sciences, College of Dental Medicine, Pomona, CA, USA

(Note: The College of Dental Medicine does not have separate departments)

Running Title: Isotretinoin and Palatal Expansion

Correspondence to:

James Borke, PhD.
Western University of Health Sciences
College of Dental Medicine
309 E. Second Street
Pomona, CA 91766
USA

Abstract: 250 words. Full Manuscript: 2,985 words

ABSTRACT

Authors: Gage CC, Feather A, Stoianovici C, Guerra C, Borke JL

Objectives: Young people exposed to high doses of 13-cis-retinoic acid (isotretinoin) are at risk for premature epiphyseal closure and other abnormal bone changes. Acne preparations containing isotretinoin are commonly used by teenagers also receiving orthodontic therapy. This investigation was designed to determine if isotretinoin would affect expansion or relapse during palatal expansion therapy in a rat model.

Material & Methods: Age and sex matched female (n=5) ~200g Sprague-Dawley rats were treated for 47 days with 2mg/kg/day of isotretenoin IP (a dosage consistent with treatment of recalcitrant cystic acne in humans), or with DMSO vehicle as a control. Impressions of the maxilla were taken before spring placement. The palate was expanded approximately 1mm using stainless steel W-archwire springs bonded bilaterally to the molars delivering 10gm force. The expansion was held for 4 weeks. Impressions were taken at debonding, and after 3 days and 7 days. The intermolar distance for each impression was digitally measured from photographic images of the impressions.

Results: Analysis using a 2 sample t-test showed increased expansion of the palate between treated and untreated animals at debonding (1.20 vs 0.96mm respectively, $p<0.03$), and greater relapse at 3 day (0.46 vs 0.31mm, $p<0.08$) and 7 day (0.44 vs 0.27mm, $p< 0.04$) relapse periods.

Conclusion: This study suggests that isotretinoin does not hinder and may increase orthodontic expansion, however, it may also increase the likelihood of relapse after palatal expansion.

Keywords: Accutane; isotretinoin; retinoic acid; palatal expansion; rat model

CLINICAL RELEVANCE

The drug 13-cis-retinoic acid or isotretinoin is a member of the retinoid family which includes compounds structurally similar to Vitamin A. Retinoids influence various phases of cellular differentiation, mineralization events, as well as protein synthesis, cell signaling, and apoptosis.

Isotretinoin has been shown to modulate keratinocyte maturation and adhesion and to reduce the size and production of sebum secreting glands (1). Due to its effectiveness, isotretinoin is the most widely used medication for treatment of recalcitrant acne vulgaris and often has a curative effect after 4-6 months of treatment (2,3).

Because orthodontic tooth movement depends on cell signaling as well as repair and remodeling of mineralized tissue, it is our hypothesis that a 4-6 month course of isotretinoin could modulate otherwise predictable orthodontic therapy. This study seeks to elucidate the effect of isotretinoin on orthodontic tooth movement.

INTRODUCTION

The standard regimen of 13-cis-retinoic acid for acne vulgaris consists of a dosage of 0.5mg - 2mg/kg/day until major outbreaks subside, generally after 4-6 months. The most serious side effect of isotretinoin is its action as a teratogen with other side effects appearing similar to those reported with hypervitaminosis A (4).

The symptoms of Vitamin A toxicity include reduced bone mineral density, calcification of tendons and ligaments, and premature epiphyseal closure. The ability of Isotretinoin to affect mineralized tissue does so in a time and dose dependent manner. It is unclear if young adults receiving a short course of low-dose isotretinoin therapy for the treatment of acne have had any damaging effect on bone mineral density (5).

The clinical significance of secondary effects of isotretinoin therapy has yet to be determined. Isotretinoin is most commonly prescribed to young adults who are actively growing and where disturbances in bone mineralization could have detrimental long term effects. Young adults are also the group most likely to initiate orthodontic treatment for the correction of various malocclusions.

MATERIAL and METHODS

Animals:
Twenty Sprague-Dawley female rats aged 6 weeks with an average body weight of 220g were used for this study. All rats were fed standard rat chow and water *ad libitum*. Animals were housed 2 per cage with a standard 12 hour light-dark cycle. IAUCUC approval was granted by Western University of Health Sciences prior to initiation of the experimental trials.

Experimental Group:
The experimental group (RA) consisted of ten rats. Animals in the experimental group received IP injections of isotretinoin at a dosage of 2.0mg/kg/day prepared in a 0.1mL solution of corn oil with 0.2µL of DMSO solvent. This dosage was consistent with the dosage used for treatment of recalcitrant cystic acne in humans. Due to the volatile nature of the drug, the oil suspension was mixed individually and kept in a dark, temperature controlled (4°C) environment until use.

Control Group:
The control group also consisted of ten rats. Animals in the control group received 0.1mL daily IP injections of corn oil containing 0.2µL of the DMSO solvent.

Isotretinoin Administration:
IP Injections to both the experimental and control groups began 7 days prior to spring placement to allow sufficient time for the drug to reach therapeutic levels in the serum. Injections were then administered daily to both groups until the study was concluded. After 4 weeks of expansion, the springs were removed and relapse was monitored at 3 and 7 days.

Orthodontic Spring Design:
In order to test the effect of isotretinoin on orthodontic expansion, custom orthodontic springs were bent from 0.012 stainless steel wire using diagnostic casts (Figures 1A & 1B). The springs were fashioned to adapt onto the lingual surfaces of the maxillary molars and deliver a buccally directed expansive force of 50 gms via 1mm of activation distributed to the 6 maxillary molars (approximately 10 gms per molar). This spring design was selected due to the relative ease of placement and the occult location on the palate. The springs were activated extraorally and a ligature was placed to restrict activation until the spring could be securely bonded intraorally (Figure 1C). Previous studies on movement of rat teeth suggest that approximately 10gms of force is sufficient to move teeth in a rat model (6).

Spring Placement:
Each animal was anesthetized using an intramuscular injection of a cocktail composed of ketamine (100 mg/mL), xylazine (20 mg/mL) and acepromazine (10 mg/mL) at a dosage of 0.5 mL/kg. While the animals were anesthetized, the spring was bonded to the lingual surfaces of the maxillary teeth. The teeth were prepared and bonded with Kerr Revolution flowable light-cured composite according to the manufacturer's instructions (Kerr, Orange CA). The spring was then activated and left undisturbed until impressions were captured (Figure 1C).

Measurement of Tooth Movement and Relapse:
Impressions were captured using a light-body non-elastomeric polyvinyl siloxane dental impression material (Kerr, Orange CA) (Figure 1D). Impressions were captured at the time of spring placement (t=0 days), at spring removal (t=28 days), and on day 3 (t=31 days) and day 7 (t=35 days) of relapse.

Interpretation of Data:

Calibrated photographs were taken of all the impressions and were analyzed using Adobe Photoshop CS5 (Extended V.12) (Adobe Systems Inc., San Jose, CA). Tooth movement was determined by measuring the inter-cuspal distance between the buccal cusps of the maxillary first molars. Only one measurement was used due to impression irregularities caused by soft tissue swelling.

RESULTS

The spring design was successful in expanding the inter-arch distance between all of the molars in the rat maxilla. Our sample size decreased to n=9 due to bond failures observed on the day of debonding.

A 2 sample t-test analysis showed increased expansion of the palate between isotretinoin treated (RA) experimental animals and untreated control animals at debonding after 4 weeks of expansion (1.20 vs 0.96mm respectively, $p<0.03$), and greater relapse at 3 day (0.46 vs 0.31mm, $p<0.08$) and 7 day (0.44 vs 0.27mm, $p< 0.04$) relapse periods (see Figure 2), though the relapse after 3 days was not statistically significant.

DISCUSSION

The use of a palatal expander in humans causes an increase in intramolar distance through a combination of orthopedic expansion, alveolar bone bending, and tooth tipping; however, the contribution of these dental and bony changes depends upon the age of the patient, suture fusion, rate of palatal expansion, force applied, and retention methods (7,8). To our knowledge, this study represents the first time isotretinoin has been used in palatal expansion on rats and is one of few studies focusing on the relationship of pharmacologic factors that may affect palatal expansion(9).

The mechanism of action of isotretinoin is unclear, but is thought to be similar to all-trans-retinoic acid; the active metabolite of Vitamin A (10). Retinoic acid interacts with nuclear receptor binding sites affecting DNA transcription and ultimately protein expression. Retinoid receptors are found in numerous cells such as liver, kidney, and bone tissue. The 13-cis retinoic acid molecule undergoes some isomerization to all-trans-retinoic acid but does not have the same affinity for nuclear retinoic acid receptors (10). The unique mechanism of isotretinoin may have some intrinsic effect on DNA transcription that has not been described in literature; however it is unclear what proportion of the isotretinoin effect on the body is actually due to isomerized retinoic acid.

Osteogenesis in the growth of long bones depends on the action of endogenous retinoids to promote and maintain chondrocyte maturation. Endogenous retinoids, chiefly all-trans-retinoic acid, enter the cytosol of chondrocytes and regulate the processes of terminal differentiation that lead to the deposition of bone on the existing cartilaginous matrix (11). The signal for beginning the process of endochondral ossification is triggered by the retinoid-induced expression of type X collagen in the hypertrophic zone in the epiphyseal region of long bones.

This specialized signal to begin osteogenesis is also joined with transcription factor RUNX2, the action of thyroid hormones, androgen production, and members of the transforming growth factor superfamily (12). Wang and Kirsch (13) also demonstrated that mature chondrocytes under the influence of all-trans-retinoic acid produce large quantities of annexins type II, V, and VI. These proteins embed themselves in the phospholipid membrane and form channels that trigger an influx of Ca^{2+} prior to matrix calcification thus underscoring the role of retinoic acid in bone formation (11,13). Therapeutic amounts of exogenous 13-cis-retinoic acid administered for a period of 6 months have been shown to cause chondrocyte hyper-maturation during endochondral ossification leading to reports of premature epiphyseal closure in children (14-16). These findings are consistent with the proposed effect of administering all-trans-retinoic acid for the same time period.

Other changes in mineralized tissue have also been documented with the use of isotretinoin. Hyperostotic changes have been reported in patients who were administered an average of 2.0mg/kg/day with radiographic change present after 6 months of treatment (17). More recently, Bergoli et al studied the effect of isotretinoin on alveolar repair in rats after exodontia. Daily doses of 7.5mg/kg of body weight p.o. of isotretinoin were reported to increase bone repair via accelerated epithelial cellular differentiation and maturity as well as increased deposition of new compact bone. (18).

Despite compelling data that supports the hypothesis that isotretinoin administration causes an increase in bone formation and repair, osteolytic changes have also been documented after the administration of 13-cis-retinoic acid. Very early studies showed that increases in dietary Vitamin A can cause bone demineralization that can lead to fracture (19). Isotretinoin administration has been documented to cause a transient decrease in serum calcium with a compensatory rise in parathyroid hormone with normal serum calcium levels returning to baseline values within 2 weeks (20). Kindmark, in 1998, could not explain the reason why isotretinoin caused a temporary drop in markers of bone turnover in acne patients (20). Wang and Kirsch's work in 2002 (13) describing the formation of an annexin mediated calcium channel which would trigger an influx of calcium into the cell thus causing a decrease in serum calcium that would be corrected by compensatory mechanisms.

Interestingly, isotretinoin is known to cause both osteoporosis and hyperostotic changes resembling diffuse idiopathic skeletal hyperostosis (DISH) (21). Early studies have suggested that high-dose, long-term isotretinoin treatment may cause demineralization and osteoporosis (21,22); however, conflicting results have been reported in studies evaluating changes in bone mineral density (BMD) with short-course isotretinoin therapy for acne (23). Leachman (24) showed that bone density at Ward's triangle decreased a mean of 4.4% after 6 months of oral isotretinoin compared to healthy age and gender matched subjects. In a multicenter study, DiGiovanna et al.(25) investigated the effects of isotretinoin therapy on BMD in 217 patients with severe, recalcitrant, nodular acne and reported similar findings. Although DiGiovanna et al. (25) and Leachman et al. (24) found a decrease in BMD in Ward's triangle of the hip, Ward's triangle can be affected by positional changes and is not considered suitable for evaluating BMD. Indeed, the World Health Organization recommends that the total BMD values obtained

from at least two different locations should be considered in order to diagnose osteoporosis (26).

CONCLUSIONS

While the specific mechanism of isotretinoin on palatal and dental expansion is unclear, our findings suggest that isotretinoin does not hinder and may increase orthodontic expansion, however, it may also increase the likelihood of relapse after palatal expansion.

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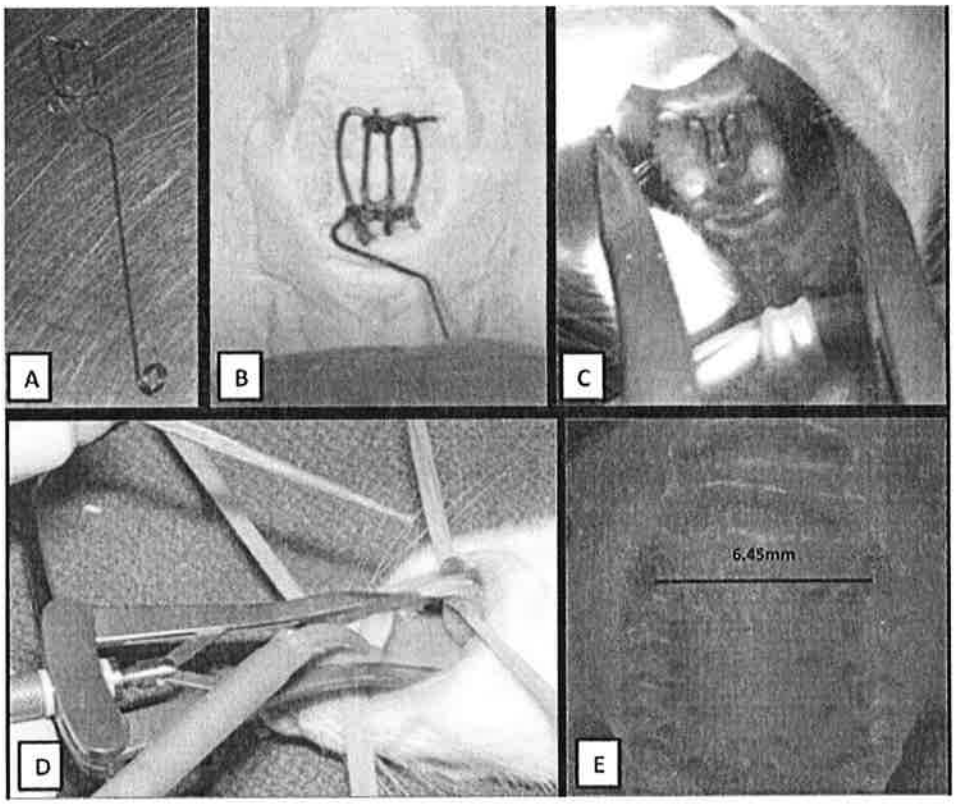


Figure 1: Palatal Expansion and Measurement. A) Orthodontic spring design; B) Preactivation using casts of the rat maxilla; C) Spring placement and bonding; D) Dental impression technique in the anaesthetized rat; E) Measurement of tooth movement and relapse using dental impressions.
179x151mm (96 x 96 DPI)

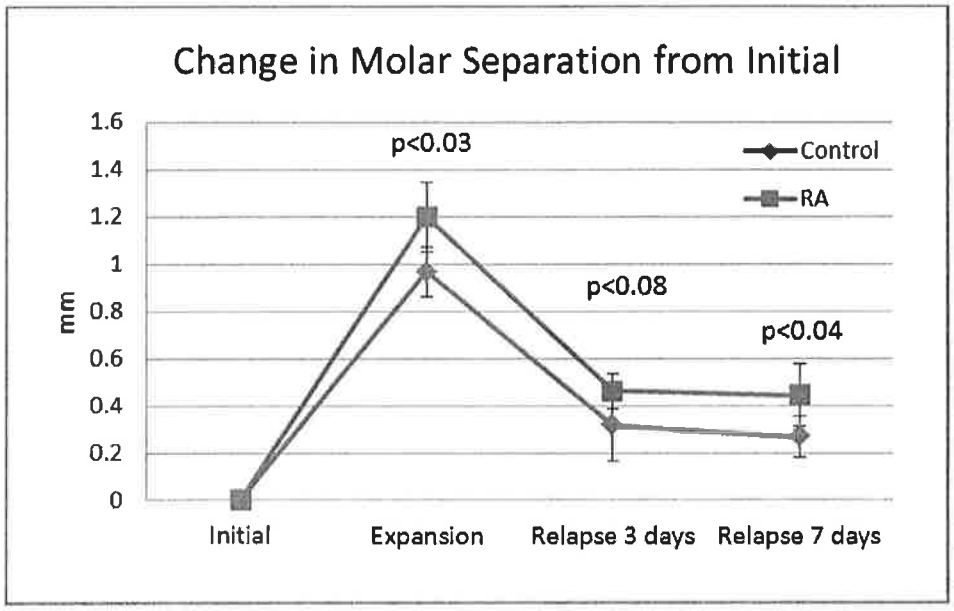
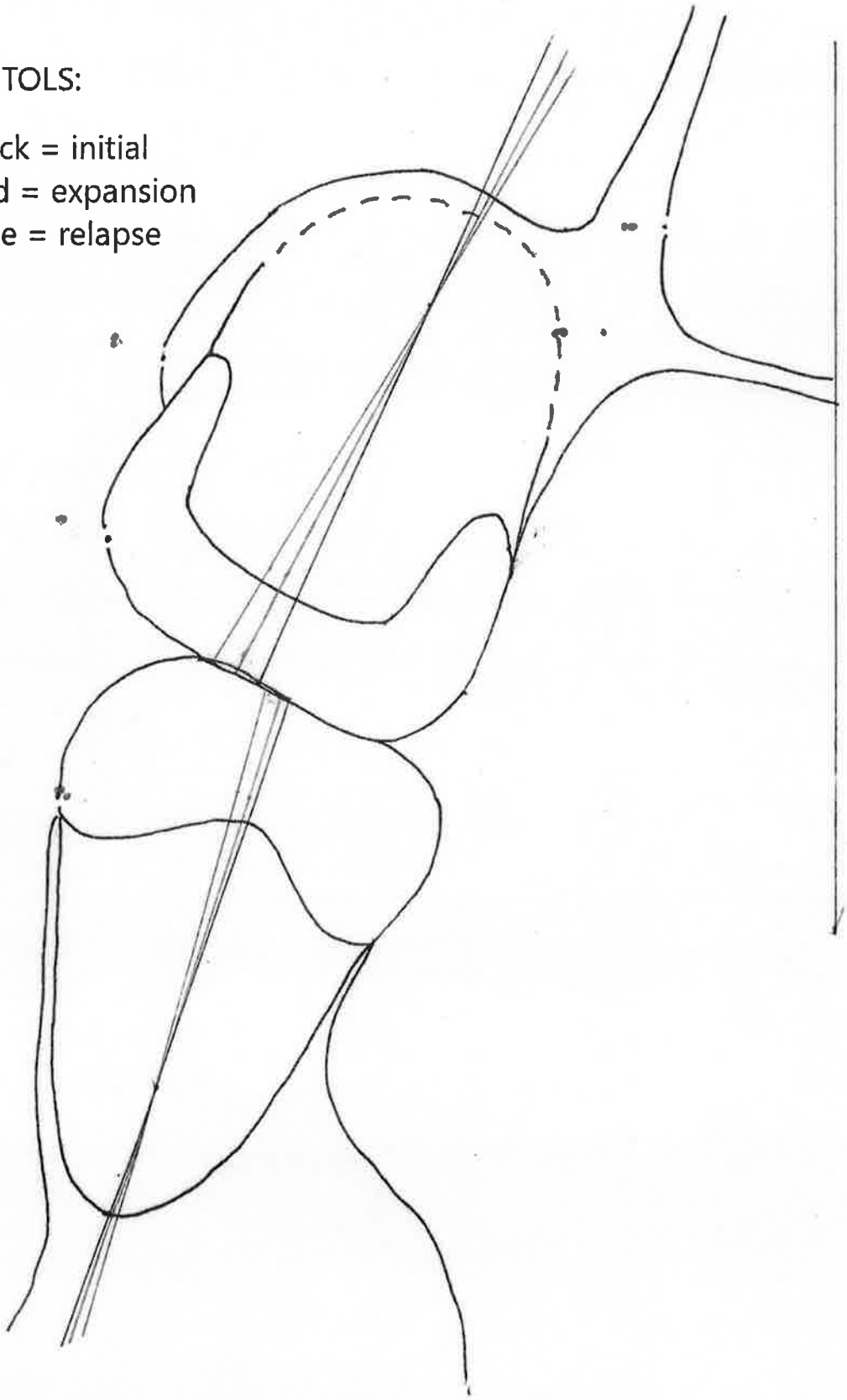


Figure 2: Change in molar separation after expansion and relapse in the presence and absence of isotretinoin.
127x81mm (150 x 150 DPI)

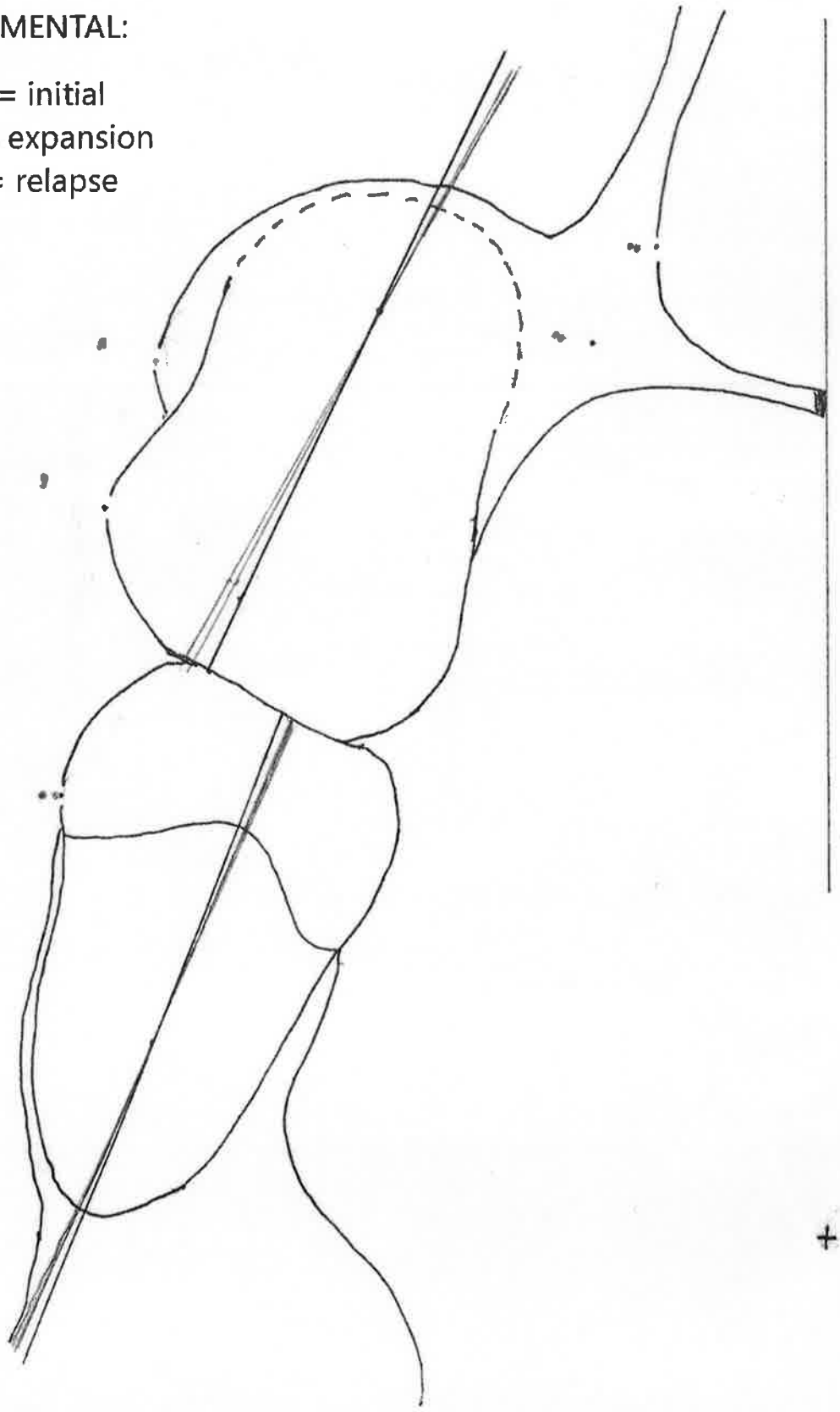
CONTOLS:

Black = initial
Red = expansion
Blue = relapse



EXPERIMENTAL:

Black = initial
Red = expansion
Blue = relapse



Superimpositions

Black = Control
Red = Experimental
Blue = Initial