Force magnitude effects upon osteoprogenitor cells during premaxillary expansion in rats

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The most critically evaluated treatment variable of maxillary expansion is the magnitude of force. Clinically this variable is observed as the expansion rate. Rapid maxillary expansion techniques usually employ a jackscrew-type of appliance and a schedule of frequent activation.[1-4] Isaacson[3] reported that a single activation of a jackscrew appliance could produce forces in the 3- to 10-lb range, while multiple daily activations could accumulate 20 lbs of force or more, depending on the age of the patient. Forces of high magnitude are thought to maximize orthopedic separation by disrupting the suture tissues before substantial lateral tooth movement can occur.[2,5] Histologic evaluation of rapid maxillary expansion reveals bone fragments, highly disorganized and vascular connective tissues, and variable bone production.[6-8] While the suture connective tissue heals with a proliferative response, a stable maxillary complex is not achieved until the residual forces which tend to collapse the expanded segments have dissipated.[1,8] Several investigators have suggested that retention periods should be longer (3 to 6 months) following rapid maxillary expansion in order to allow suture reorganization and to minimize skeletal relapse.[1,9-11]

Slow maxillary expansion involves forces varying from ounces to several pounds. Both tooth

Abstract

To study the effects of force magnitude on osteoprogenitor cell activity during premaxillary expansion, stainless steel helical springs were attached to the maxillary central incisors of 45 3-month-old male rats. The animals were randomly divided into force levels (0, 50, 100, 150, 200 gm) and were injected intraperitoneally with tritiated thymidine (1.0 uc/g wt.) 1 hour prior to sacrifice which occurred at 27, 40, and 60 hours. In order to examine cell activity within different regions of the suture, each premaxilla was divided into three geographic areas. Quantitative results were obtained by comparing the percent of labeled cells observed at different force levels, geographic areas, and observation times. The greatest number of labeled cells at each force level was found at 27 hours. Increased forces were correlated with increased numbers of labeled cells up to 100 gm, with decreased cell numbers at higher forces. The numbers of labeled cells at 200 gm were not significantly different from the controls. Histological observations of early bone formation at 60 hours supported the quantitative labeling results at 27 hours. The results also demonstrate a significant correlation between the geographic location of the labeled cells and force magnitude, with maximal cell stimulation occurring more superiorly in the suture as forces increased. The results suggest that early bone formation within the expanded suture can be maximized by varying force magnitude and distance from the point of force application.

This manuscript was submitted February 1991. It was revised and accepted August 1991.

Key Words

Maxilla • Expansion • Force • Osteoprogenitor cells • Rat

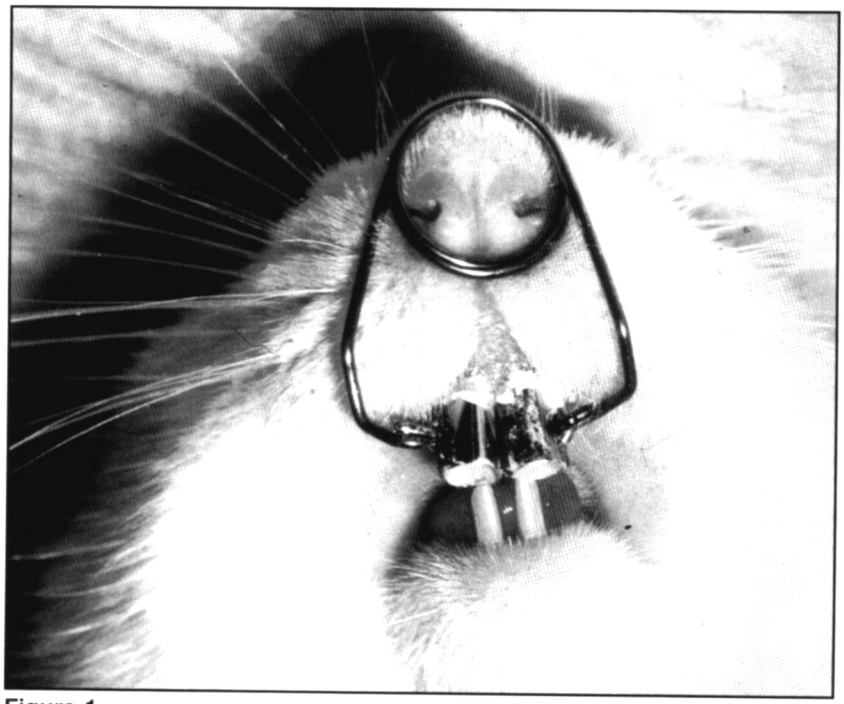

Figure 1

Figure 1
Maxillary expansion appliance in place.

Figure 2A
The premaxilla sectioned tangential to the plane of the incisors at the midpoint.

Figure 2B
A section showing the superior, middle, and inferior areas of the interpremaxillary suture. (S = interpremaxillary suture.)

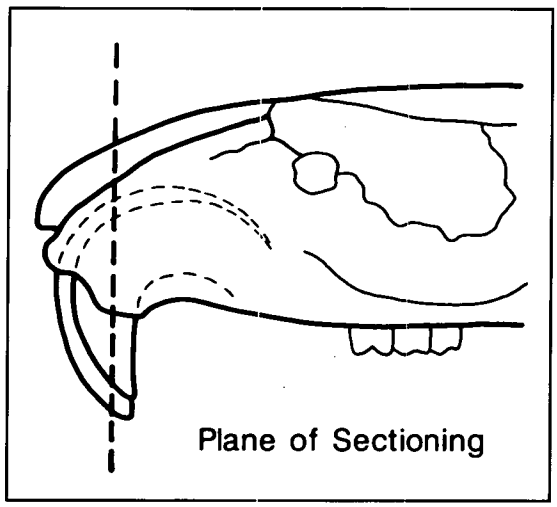

Figure 2A

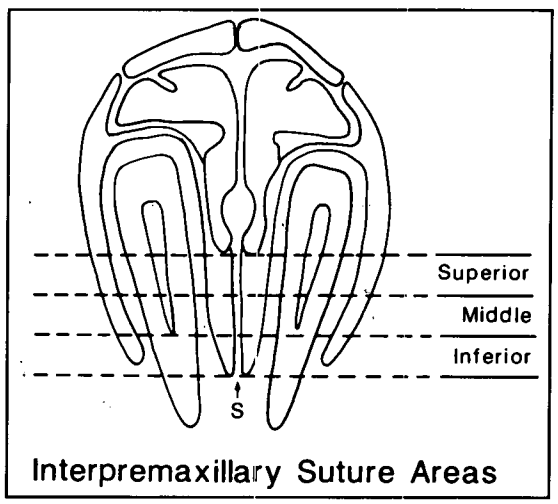

Figure 2B

movement and orthopedic separation are reported to occur with this method.[8,9,12-14] Hicks,[14] using a 2-lb expansion force in children estimated 30% of the total change to be skeletal. Histologic findings suggest suture widening should occur at a rate which maintains the integrity of the sutural tissue.[7,9,12,15] Ekstrom et al.[1] reported that slowly expanded sutures became well organized with mineralized tissue in 1 to 3 months. Storey[7,9,15] suggested slow premaxillary expansion of 0.5 to 1.0 mm per week allows physiologic separation of the suture with less trauma, better healing, and greater stability than observed with rapid expansion techniques. Storey[7] also suggested that an optimal expansion force exists, which could maximally stimulate bone production. Clinical observations have indicated skeletal relapse may be reduced following slow maxillary expansion, requiring a retention period of less than 3 months.[1,3,9,12,14,16,17]

Although force magnitude is observed to affect the amount of bone growth within the premaxillary suture during expansion,[7,8] research has not demonstrated a quantitative relationship between applied force and osteogenesis. The purpose of this study is to investigate the effect of force magnitude upon labeled osteoprogenitor cells within the interpremaxillary suture following premaxillary expansion in rats.

Materials and methods

Forty-five male Sprague Dawley rats, weighing 280±10 gm were used in this study. The rats were acclimatized for 1 week with the lights adjusted to a 12-hour day/night cycle. The animals were randomly divided into 15 groups of 3 rats. The 15 groups consisted of 5 expansion force levels (control, 50, 100, 150, 200 gm) and 3 durations of expansion (27, 40, 60 hr).

Helical springs were constructed to deliver the desired force level ± 10% over an opening span of 6.0 mm, measured by a 200 gm tension gauge in vitro. The spring appliance was fixed by placement through the lateral eyelets after band cementation (Figure 1). The rats were immobilized by intraperitoneal injection of pentobarbital (5 mg/100 gm weight) prior to the banding procedure.

Each rat was injected intraperitoneally with tritiated thymidine, 1.0 uCi/gm weight, in order to label the DNA of active mitotic cells. All animals were sacrificed 1 hour after injection to limit cell division and dilution of the label.

The rats were euthanized with pentobarbital (15 mg/100 gm weight), followed by an intravenous injection of 10% buffered formalin for immediate tissue fixation. The premaxillary regions were dissected out and placed into 10% buffered formalin

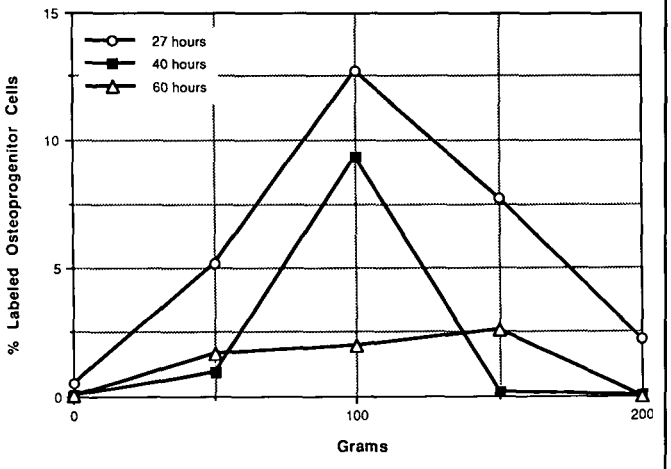

Table 1
The mean percent of labeled cells observed at each expansion force and time within the entire suture.

	Force (gm)				
Hours	0	50	100	150	200
27	0.53	5.19	12.69	7.69	2.22
SD	0.65	2.61	3.48	4.13	2.97
40	0.08	0.97	9.31	0.19	0.06
SD	0.28	0.74	8.49	0.52	0.23
60	0.47	1.69	1.97	2.58	0.00
SD	0.56	1.31	1.56	2.26	0.00

Figure 3

for 3 days to ensure proper fixation. After 5 weeks of decalcification in 10% buffered formic acid, the anterior regions of the premaxilla were placed into paraffin blocks and cut tangentially to the plane of the incisors. Five serial 6-micron sections were taken of each premaxillary suture (two for histology and three for autoradiography) at the midpoint of the maxillary incisor (Figure 2A). For histological examination, the sections were stained with hematoxylin and eosin or with Mallory's trichrome.

The autoradiographic technique consisted of dipping premounted slides subbed with gelatin into a photographic emulsion (Kodak NTB 2). The slides were placed in a light-proof box and stored in a 4°C refrigerator for 4 weeks. They were developed (Dektol/H20 1:1), for 2 minutes, fixed (Kodak regular) for 5 minutes and lightly stained with hematoxylin and eosin to define the nuclei within the cells. Slides were examined at random. The radioisotope uptake into the DNA was recorded by observing black granules within the developed photographic emulsion. A labeled osteoprogenitor cell was defined by 15 or more black granules appearing over the nucleus. The osteoprogenitor cell was characterized as a fibroblast-like cell near the osseous border with pale staining nucleus of a diameter 5-7μm. By direct observation through a 400X microscope, labeled and nonlabeled osteoprogenitor cells were counted and a mean percent of labeled osteoprogenitor cells (hereafter called labeled cells) was calculated.

Each area within the premaxillary suture was defined as a linear measure of 5 consecutive 100 micron standardized grids (Figure 2B). The mean percent of labeled cells counted in 5 grids was defined as one data point.

The results were quantitated by comparing the mean percent labeled cells observed at different force levels, geographic areas of the suture, and observation times. Means, standard deviations and p levels of significance were calculated by the UCLA Department of Statistics using the P4V program in the BMDP statistical software package.

Results

The expansion time was critical to the evaluation and comparison of labeled cells. The highest percentage of labeled cells was found after 27 hours of expansion (Figure 3, Table 1). Only at 27 hours were comparisons of labeled cells found to be significantly different between force levels. (Table 2).

The data showed that the magnitude of the expansion force significantly affected the percentage of labeled cells. As the expansion force increased, cell labeling initially increased, peaked at 100 gm, and decreased thereafter (Figure 3). The percentage of labeled cells observed with 50 gm and 150 gm of force was significantly greater than the percentage seen in the control group, but significantly lower than that seen in the group which received 100 gm of force (Table 2). The 200 gm force group showed few labeled cells with no significant difference when compared to the control group. Histologic observations of the suture showed increased stretching of collagen fibers with force until complete severance occurred at 200 gm. At 60 hours, the amount of early

Figure 3
The mean percent of labeled cells was observed at each expansion force and time within the entire suture. Most of the labeled cells were observed at 27 hours. As the expansion force increased, the labeled cells increased, peaked at 100 gm, and then decreased.

Table 1
The mean percent of labeled cells observed at each expansion force and time within the entire suture. (The data points with standard deviations plotted in Figure 3).

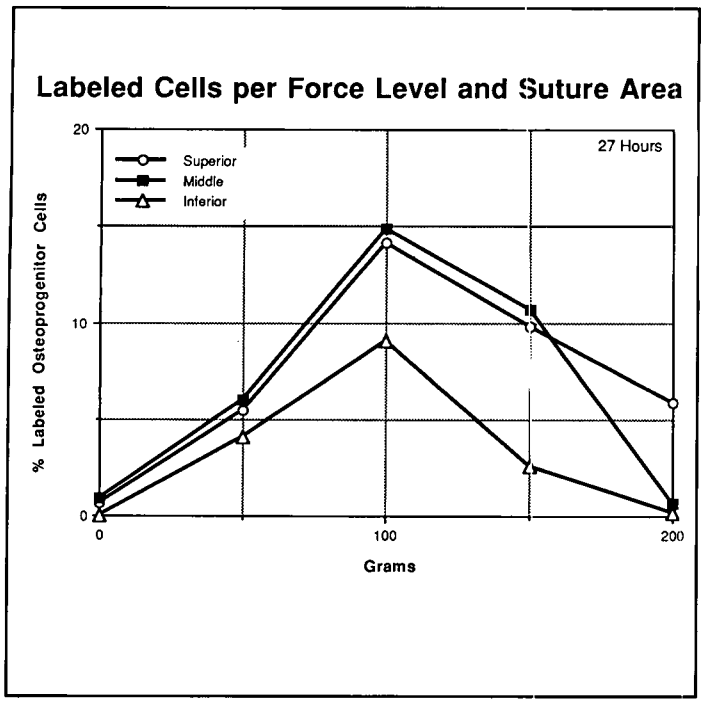

Figure 4

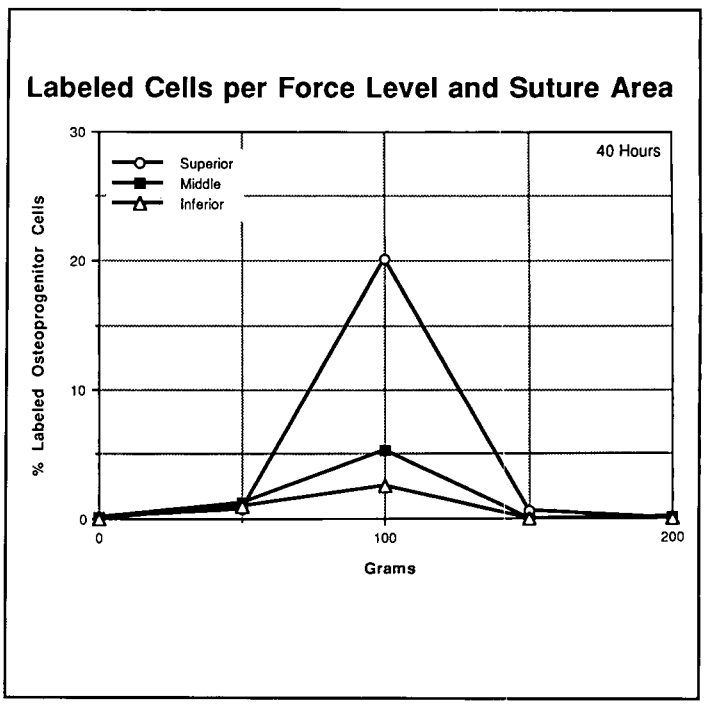

Figure 5

Figure 4
The mean percent of labeled cells observed with each expansion force and within each suture location. At 100 and 150 gm, an increase of labeled cells was seen within the middle and superior areas when compared to the inferior area. At 200 gm, an increase of labeled cells occurred only within the superior area when compared to the middle and inferior areas.

Figure 5
At 40 hours, the mean percent of labeled cells observed with each expansion force and within each suture location. Only one significant peak occurred, within the superior area at 100 gm of expansion force.

bone formation observed supported the quantitative labeling data at 27 hours. The most bone formation was noted at the 100 gm force level with lesser amounts at 50 gm and 150 gm. No bone formation was observed in the control or 200 gm level animals.

With an expansion force of 100 gm or greater, the data showed cell labeling was significantly dependent upon the location of the suture observed (Figure 4 and Table 3). Both the 100 gm and 150 gm forces showed an increase of labeled cells within the superior and middle areas compared to the inferior area. Yet, an examination of the 200 gm force revealed a significant increase of labeled cells within the superior area in comparison to the middle and inferior areas.

As stated earlier, the expansion times of 40 and 60 hours did not reveal adequate labeled cells to provide significant comparisons between force magnitudes, except at at 40 hours and 100 gm of force (Figure 3). The 100 gm expansion force revealed significantly more labeled cells with the increase occuring mainly within the superior area of the suture (Figure 5).

Discussion

The results show that the mean percent of labeled cells is dependent upon the magnitude of force expanding the rat interpremaxillary suture. As the expansion force increases, labeled cells increase, peak at 100 gm, and decrease thereafter (Figure 3). These results suggest the presence of an optimal expansion force which can maximally stimulate cell proliferation and subsequent bone formation. The direct relationship between the proliferation rate of osteoprogenitor cells and early bone formation has been previously reported.[19-22] However, controversy exists over the presence of an optimal premaxillary expansion force within the rat model.[7,8,23,24] Morndal[23] found labeled cells increased and reached a plateau with premaxillary expansion forces of 20 to 35 gm. Morndal[25] stated the major force decline in the spring appliance occurred within the first 24 hours. It is possible force magnitude became too small to produce dissimilar percentages of labeled cells. In contrast our study used greater intervals between forces (0, 50, 100, 150, and 200 gm). Our data does not reflect a plateau of labeled cells, but rather an initial increase in labeling with force and then a decrease as the force increased beyond 100 gm. Southard and Forbes[24] found no difference between labeled cells when they compared low (50 to75 gm), medium (150 to 175 gm) and high forces (250 to 300 gm). A spring designed with short arms and a small helix diameter may have provided a rapid decline of the high force within the first 24 hours as lateral movement occurred. Our expansion appliance was designed with long arms and a wide helix diameter so the spring could deliver the measured force ± 10% as it opened 6 mm. The highest force, 200 gm, expanded the teeth 4 mm during the study duration of 60 hours, assuring the force delivered was within the measured range.

Interestingly, there were no significant differences between labeled cells observed at the 200 gm force and zero force (Table 2). Collagen fiber tearing

Table 2
p levels of significance comparing percent of labeled cells observed with each force level at 27 hr

Force (gm)	Force (gm) 50	100	150	200
0	0.0014	<0.0001	0.0001	0.1445
50		<0.0001	0.0417	0.0196
100			0.0009	<0.0001
150				0.0005

Table 3
Mean percent labeled cells observed with each expansion force within each suture location at 27 hr

Area	Force (gm) 0	50	100	150	200
Sup	0.67	5.50	14.17	9.83	5.83
SD	0.78	2.54	2.55	2.04	2.33
Mid	0.92	6.00	14.83	10.67	0.67
SD	0.51	2.17	2.66	2.42	0.98
Inf	0.00	4.08	9.08	2.58	0.17
SD	0.00	2.87	1.83	1.00	0.39

increased as the expansion force increased above 100 gm. At the 200 gm level, the fibers were completely broken with no early bone formation observed. However, high force expansion of the suture may disrupt vascular delivery of the radionuclide and may decrease labeling of the mitotic cells. The mechanical disruption of collagen fibers appears to be the likely cause for the decrease in bone formation. Different theories have been presented as to how a mechanical force can be translated into bone production.[20,23,26] The transduction of mechanical force into a biological response may involve an intracellular influx of Ca and/or Na ions which decreases cAMP and triggers DNA synthesis and cellular proliferation.[20] Ten Cate et al,[27] used high forces to expand cranial sutures and showed fibroblasts must proliferate and repair sutural connective tissue before osteogenesis and remodeling of the suture takes place.

The labeled cells depend upon not only the force magnitude, but also the location observed within the premaxillary suture. The 100 and 150 gm expansion forces showed more labeled cells within the middle or superior areas than the inferior area, suggesting the optimal force was exceeded within the inferior area (Figure 4). Histologically, the inferior areas had many collagen fibers torn from their insertions while the middle and superior areas had stretched, but intact fibers. The 200 gm expansion force showed significantly more labeled cells within the superior area than the middle or inferior areas, suggesting the optimal force is exceeded within the inferior and middle areas. Histologic observation confirmed only the superior area had intact collagen fibers, while the remainder of the suture demonstrated fibers torn from their insertions. The results suggest the expansion force dissipates within the interpremaxillary suture from an inferior to a superior direction.

The clinical rationale for rapid palatal expansion is to produce orthopedic movement of the maxilla before there is time for significant tooth movement to occur. Unfortunately this type of expansion must be retained until the suture fills in with new bone and the maxillae stabilize. During retention the two halves of the maxilla may relapse medially while the teeth are being held in place by the appliance; in effect, by orthodontic tooth movement. The amount of tooth movement that occurs may be as great or greater than that produced with slow expansion techniques. By allowing new bone production to occur at a maximal rate, i.e. by using an optimal force, treatment time may be shorter, with more mature bone and greater stability occurring sooner.

Only one result outside the 27 hour observation is noteworthy. After 40 hours, the 100 gm expansion force labeled more cells within the superior area of the suture than any of the other force levels or suture locations (Figure 5). This suggests the cells may undergo a maximal second phase of mitosis when optimal force is achieved within a specific site. Various authors reported multiple mitotic phases of osteoprogenitor cells occurring from mechanical stimulation. Roberts[28] reported two phases of mitosis (before 16 hours and between 30 to 50 hours) within the periodontal ligament of the

Table 2
p levels of significance comparing the percent of labeled cells observed with each force level at 27 hours throughout the entire suture. Note the high level of significance between force levels obtained except the comparison between the control and the 200 gm.

Table 3
At 27 hours, the mean percent of labeled cells was observed with each expansion force and within each suture location. (The data points with standard deviations plotted in Figure 4).

rat. Baumrind et al.[29] found labeled cells to peak at 24 and 54 hours in the periodontal ligament. Morndal[25] may not have observed a maximum labeling of cells at 40 hours because of the low magnitude of expansion force used.

This research suggests an optimal premaxillary expansion force leads to maximal early sutural bone formation. Since both high and low expansion forces eventually result in complete sutural bone production, the question remains, "Would maximal early bone production significantly lessen relapse or retention time?"

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J.J. Zahrowski submitted this paper in partial fulfillment for the degree of Master of Science in Oral Biology, University of California at Los Angeles, School of Dentistry.

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