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Orthodontic force decreases the eruption rate of rat incisors

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SUMMARY The aim of this study was to determine whether a force applied in an antero-posterior direction would adequately reduce incisor eruption. This is needed to achieve a constant direction of force which is one of the demands for a good model for studying orthodontic tooth movement. Twenty male Wistar rats aged 11–12 weeks were divided into two equal groups: in the appliance group, a superelastic closed coil spring (25 cN) was placed between the upper left first molar and the incisors. The control group consisted of animals without an appliance. In both groups, cuts were created on the labial surfaces of the upper and lower incisors. The distance from the gingival reference point to the midpoint of the cut was measured for 10 days at 2 day intervals. Upper incisor inclination was determined as the distance from the most mesial point of the upper left first molar to the incisal edge of the ipsilateral incisor on days 0 and 10. Statistical analysis was carried out using two-way analysis of variance and a Bonferroni post- test to estimate reliability.

The eruption rates of the maxillary incisors in the appliance group were significantly decreased when compared with the control group during the whole experiment. In the appliance group, the eruption rates of the mandibular incisors were decreased more than those of the maxillary incisors ($P < 0.01$). There was no difference in incisor inclination between the appliance and control groups on day 10 ($P = 0.81$). The applied force of 25 cN in an antero-posterior direction diminished incisor eruption to a level which enabled a constant direction of orthodontic force for 10 days.

Introduction

Both clinicians and researchers have observed individual differences in the amount and rate of orthodontic tooth movement (OTM) despite the same force systems being used (Pillon *et al.*, 1996; van Leeuwen *et al.*, 1999; von Böhl *et al.*, 2004). Biological responses to force application are complex. To understand the biological mechanisms, animals have been widely used in experimental studies of OTM (Pillon *et al.*, 1996; Guajardo *et al.*, 2000; Verna *et al.*, 2004; de Carlos *et al.*, 2006). Different animal species have been used (dogs, cats, monkeys, and pigs), but the use of rats predominates. They are considered to be suitable for studying OTM (Ren *et al.*, 2004). A good animal model has to meet the following demands: a constant direction and action of orthodontic force, a constant and adequate orthodontic force magnitude, and an accurate method of measuring tooth displacement. The continuous eruption of rat incisors is a physiological aspect which should be considered in establishing a good rat model for studying OTM. It can change force direction and cause a loss of anchorage (Ren *et al.*, 2004).

Rat incisors grow continuously, displaying the complete life cycle of tooth development from inception to maturity (Schour and Massler, 1949). Several studies have examined the influences on the eruption rates of rat incisors, and in some of them, the role of shortening of the rat incisors on their eruption rates had been evaluated (Burn-Murdoch, 1995, 1999; Risnes *et al.*, 1995; Moxham, 2002; Law *et al.*, 2003). It has been suggested that the eruption rates are determined by the sum of the lengths of the upper and lower incisors, rather than by their lengths (Burn-Murdoch, 1999). There is no effect of the consistency of the diet on the eruption rates and lengths of rat incisors (Burn-Murdoch, 1993). It has also been found that a vertically applied force diminishes or completely stops the eruption of rat incisors (Burn-Murdoch, 1981; Chiba *et al.*, 1996; Shimada *et al.*, 2003).

To compensate for incisor eruption in a rat model, Ren *et al.* (2004) suggested drilling a transverse hole through both the alveolar bone and the maxillary incisors at the mid-root level. Using this method, they found that eruption of incisors was completely stopped (Ren *et al.*, 2004). Most other studies of OTM did not take incisor eruption into consideration (Ong *et al.*, 2000; Jäger *et al.*, 2005; de Carlos *et al.*, 2006; Mermut *et al.*, 2007).

One of the demands for a good rat model for studying OTM is a constant direction of orthodontic force. The aim of the present study was to determine whether a force of 25 cN applied to the teeth in an antero-posterior direction would adequately reduce incisor eruption in order to establish a good model for studying OTM in rats.

Materials and methods

The research was approved by the Veterinary Administration of the Republic of Slovenia (No. 323-02-234/2005/2).

Twenty male Wistar rats, aged 11–12 weeks and weighing from 300 to 340 g, were used. The rats were housed in groups of five in polycarbonate cages (Ehret, Emmendingen, Germany) with 12 hour circadian conditions. The temperature and humidity were maintained at constant levels of 24 ± 1°C and 60 to 75 per cent, respectively. The animals were fed on a standard laboratory rodent diet chow (KZ Krka, Novo mesto, Slovenia), which was soaked to facilitate food intake, and took water *ad libitum* from a standard bottle with a metal delivery tube. The animals were divided into two groups (n = 10): in the appliance group, a superelastic closed coil spring was placed between the upper left first molar and the upper incisors. The control group consisted of animals without an appliance.

Placement of the appliance was performed under general anaesthesia induced by a mixture of anaesthetics injected intraperitoneally: ketamine (Bioketan, Vetoquinol Biowet, Poland, 50 mg/kg), medetomidin hydrochloride (Domitor, Orion Pharma, Finland, 67 μg/kg), and thiopental (Tiopental, Pliva, Croatia, 8.3 mg/kg). This mixture ensured 15–20 minutes lasting anaesthesia, with no spontaneous muscle spasms and with no influence on the depth or frequency of breathing.

The orthodontic appliance consisted of a superelastic closed coil spring (25 cN, wire diameter 0.15 mm, eyelet diameter 1.5 mm, GAC International, Bohemia, New York, USA), which was placed between the upper left first molar and the upper incisors. The closed coil spring was attached to the upper left first molar by a stainless steel ligature wire (diameter 0.25 mm, Dentaurum, Ispringen, Germany) and to the upper incisors by surgical steel wire (4-0, multifilament, W310, Ethicon, Johnson & Johnson, New Brunswick, New Jersey, USA). To enhance fixation of the spring to the incisors, a hole was drilled with a stainless steel round bur (diameter 0.5 mm, Meisinger, Neuss, Germany) through the approximal surfaces of the maxillary incisors just above the lateral gingival margin, and the surgical steel wire was placed through the perforation. To diminish irritation of the soft tissues, a light-curing bonding material (Tetric Flow, Ivoclar Vivadent, Schaan, Lichtenstein) was used to cover the ligature and the surgical steel wire (Figure 1).

The superelastic properties of the material and the delivered force were tested on 10 randomly selected closed coil springs from a box of 20 springs. The springs were activated from 1–20 mm. The reproducible force of 25 cN was shown over a range of 3–8 mm activation (Drevenšek *et al.*, 2006).

After the closed coil spring was attached to the teeth in the appliance group, cuts were created on the labial surfaces of the upper and lower incisors in both groups under general anaesthesia. The incisions were made at the level of the interdental papilla with a diamond disc (Horico Diamond Disk 346/90, Gresco Products Inc., Stafford, Texas, USA; Lee *et al.*, 2002). The lowest point of the labial gingiva on each incisor was used as a reference point. The distance

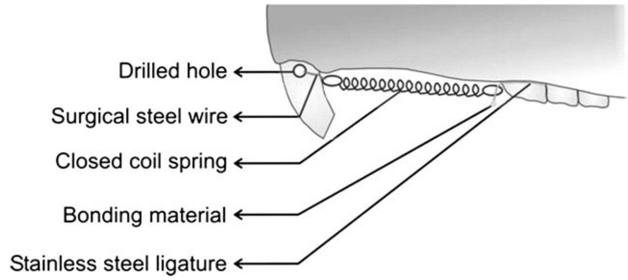

Figure 1 Schematic view of the orthodontic appliance. A superelastic closed coil was placed between the upper left first molar with stainless steel ligature and around the incisors with surgical steel wire. To enhance fixation of the spring to the incisors, the maxillary incisors were drilled with a stainless steel round bur just above the lateral gingival margin and the surgical steel wire was placed through the perforation.

from the gingival reference point to the midpoint of the cut was measured for 10 days at 2 day intervals, using calibrated graticules in microscope eyepieces. The eruption rates were calculated as the distance the cut mark travelled per day (Lee *et al.*, 2002). To test whether upper incisor inclination had changed, the distance from the most mesial point of the upper left first molar to the incisal edge of the ipsilateral incisor on days 0 and 10 was measured using a digitronic calliper with an accuracy ±0.01 mm (144-15D, Wilson & Wolpert, Utrecht, The Netherlands). All measurements were recorded twice by two investigators (ŠS and JV). Reliability was assessed using the intraclass correlation coefficient (ICC).

Statistical analysis was performed using two-way analysis of variance in GraphPad Prism 4.0 (GraphPad Software, San Diego, California, USA). A Bonferroni post-hoc test was used to estimate the reliability interval. Significance was set at $P < 0.05$.

Results

Reliability of measurements was within the standards; the mean values of the ICC for all measurements of incisor eruption rates and incisor inclination were 0.926 and 0.934, respectively. Therefore, the mean value of all measurements was used for further statistical analysis.

In the 10 day interval, the upper incisors in animals of the control group erupted 3.75 ± 0.03 mm and the lower incisors 4.86 ± 0.05 mm. In the appliance group, the eruption rates were 2.65 ± 0.03 and 2.12 ± 0.04 mm, respectively.

The eruption rates of the incisors from days 0 to 10 are given in Table 1. The eruption rates of both the upper and the lower incisors in the appliance group were significantly less at all time points except for the upper left incisor on day 8 compared with the control group.

Table 2 shows the measurements of incisor inclination (distance from the most mesial point of the upper left first molar to the incisal edge of the ipsilateral incisor) on days 0 and 10. There was no significant difference in incisor

Table 1 Eruption rates of the rat incisors. The results are given as the mean ± 1 standard deviation in millimetres per day. Significant differences between the control and appliance groups are marked in 2 day intervals.

Days	Control group	Appliance group
Maxillary right incisor		
0–2	0.42 ± 0.05	0.26 ± 0.05***
2–4	0.40 ± 0.03	0.24 ± 0.08***
4–6	0.36 ± 0.05	0.25 ± 0.05**
6–8	0.35 ± 0.05	0.25 ± 0.09**
8–10	0.36 ± 0.10	0.27 ± 0.11*
Maxillary left incisor		
0–2	0.41 ± 0.03	0.28 ± 0.07***
2–4	0.39 ± 0.02	0.25 ± 0.08***
4–6	0.38 ± 0.03	0.28 ± 0.05**
6–8	0.31 ± 0.05	0.23 ± 0.12
8–10	0.37 ± 0.05	0.34 ± 0.25*
Mandibular right incisor		
0–2	0.52 ± 0.04	0.18 ± 0.05***
2–4	0.54 ± 0.05	0.26 ± 0.08***
4–6	0.51 ± 0.07	0.19 ± 0.08***
6–8	0.43 ± 0.06	0.18 ± 0.07***
8–10	0.44 ± 0.07	0.25 ± 0.19***
Mandibular left incisor		
0–2	0.51 ± 0.05	0.20 ± 0.05***
2–4	0.57 ± 0.06	0.26 ± 0.08***
4–6	0.50 ± 0.06	0.25 ± 0.04***
6–8	0.44 ± 0.07	0.15 ± 0.08***
8–10	0.39 ± 0.11	0.21 ± 0.15***

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 2 Measurements of incisor inclination (D—from the most mesial point of the upper left first molar to the incisal edge of the ipsilateral incisor).

	Distance, D (mm)		
	Appliance group	Control group	Significance
Day 0	14.47 ± 0.30	14.56 ± 0.36	$P = 0.99$
Day 10	14.75 ± 0.29	14.74 ± 0.33	$P = 0.81$

inclination between the appliance and control group on day 0 ($P = 0.92$) or on day 10 ($P = 0.81$).

The mean eruption rates of the upper and the lower incisors during the whole period of the experiment are shown in Figure 2. There were no significant differences in the eruption rates between the left and the right incisor in either jaw within each group, but the eruption rate of the incisors in the control group was significantly greater in the upper and lower jaws when compared with the appliance group ($P < 0.001$).

The differences in eruption rates of the incisors (eruption rate of the incisors in the control group minus eruption rate of the incisors in the appliance group) in the upper and the lower jaw were compared. The differences in the eruption rates between the control and appliance group in the lower jaw were significantly greater during the whole experiment compared with the differences in the upper jaw between the control and appliance group (Figure 3).

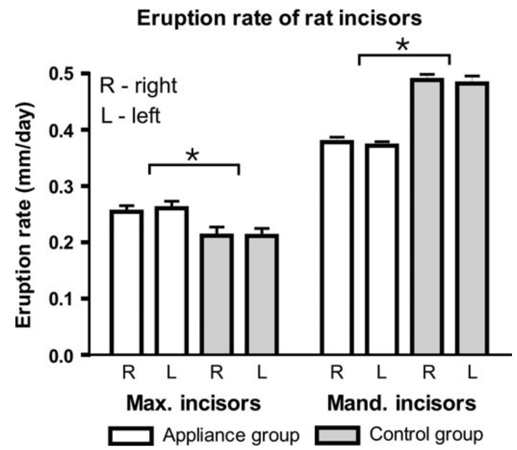

Figure 2 Mean eruption rates of the maxillary and mandibular incisors. The results are shown as the mean with standard error bars in millimetres per day. $*P < 0.001$.

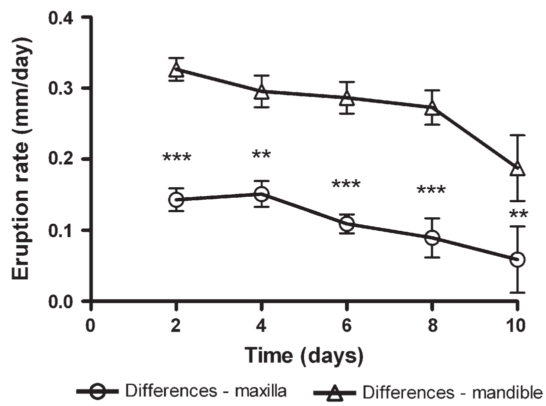

Figure 3 Differences between the eruption rates of the incisors in the appliance and control groups in the maxilla and the mandible. The results are given as the mean with standard error bars in millimetres per day. $***P < 0.001$, $**P < 0.01$.

Discussion

The eruption rates of the incisors in the appliance group, where a 25 cN closed coil spring was attached between the upper left first molar and the incisors, were significantly lower than in the control group of rats with no appliance. In the upper jaw, the incisor eruption was reduced to 1.8 mm per week. No significant differences in incisor inclination between the appliance and the control group were found during the 10 day experiment. This means that the displacement of the anchorage was negligible and that 1.8 mm of incisor eruption per week meets one of the demands for a good animal model for studying OTM, i.e. a constant direction of orthodontic force.

The force of eruption is generated in the periodontal ligament (PDL; Weinreb *et al.*, 1997). It has been found that both systemic arterial blood pressure and tissue pressure change in the PDL are both involved in determining the position of the teeth (Yamaguchi *et al.*, 1997; Shimada *et al.*, 2004; Harari *et al.*, 2005). It is assumed in the present study that during OTM, the applied force compressed the blood vessels in the PDL, resulting in a decrease of regional blood flow. This could be followed by a decrease in fluid volume and then a reduction of either pressure within the socket or the eruptive force (Shimada *et al.*, 2004).

The force that restrained the eruption of the maxillary upper incisors was equally efficient for both incisors as there was no significant difference between the maxillary left and right incisors. The upper incisors were connected to each other by a surgical steel wire placed through the perforation. The control group showed similar incisor eruption rates as were found by other researchers using a similar recording method (Burn-Murdoch, 1995, 1999; Law *et al.*, 2003; Harari *et al.*, 2005). The force induced by the closed coil spring applied to the maxillary incisors acted in an antero-posterior direction but one of the force components could also act in the vertical direction. In some studies, the amount of vertical force which could stop incisor eruption was measured. The magnitude of force needed to stop eruption was estimated to be 1.3–13.7 mN (Burn-Murdoch, 1981), 9.1–9.8 mN (Chiba *et al.*, 1996), and 2.9–4.2 mN (Shimada *et al.*, 2003). In the present study, the incisors in the appliance group were still erupting despite the small vertical force needed to halt incisor eruption. It can therefore be concluded that the appliance delivered the force predominantly in a horizontal direction and the vertical component was negligible.

The closed coil spring in the upper arch affected the incisor eruption rates even more in the lower than in the upper arch (Figure 3). This is comparable with the results of Burn-Murdoch (1999) where it was found that slow eruption rates of incisors in one jaw were associated with slow eruption rates of incisors in the other jaw. That author also reported that occlusal forces slow eruption, and the lengths of the teeth alter either the magnitude or the effectiveness of the forces (Burn-Murdoch, 1999). In the present investigation, it was assumed that placement of the appliance changed the magnitude of the occlusal forces and therefore the eruption rate of the mandibular incisors was also affected.

The results of this study show that this rat model fulfils the demand for a constant direction of orthodontic force, which is needed for a good rat model for studying OTM. The eruption rates of the upper incisors in animals with the closed coil spring were minimized to the amount needed to enable a constant direction of orthodontic force. Drilling of a hole through the maxillary incisors just above the lateral gingival margin is needed to ensure correct positioning of the spring. To maintain the position of the spring, it is sufficient to perform the drilling on a weekly basis, when measurements of OTM are usually carried out. The procedure where the eruption rate of the incisors is stopped by connecting incisor roots with the wire placed through the hole which is drilled in the apical third of the roots could lead to the release of mediators which can affect the mechanism of OTM (Ren *et al.*, 2004).

Conclusion

Orthodontic force applied between the upper left first molar and the maxillary incisors diminished the eruption rates of the incisors. The eruption rates were even more affected in the lower jaw, where there was no appliance. The eruption rates of the upper incisors in animals with a closed coil spring were reduced to an amount that enabled a constant direction of orthodontic force for 10 days.

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References

Burn-Murdoch R A 1981 The effect of applied forces on the eruption of rat maxillary incisors. Archives of Oral Biology 26: 939–943

Burn-Murdoch R A 1993 The effect of the consistency of the diet on eruption rates and lengths of incisor teeth in rats. Archives of Oral Biology 38: 699–706

Burn-Murdoch R A 1995 The effect of shortening incisor teeth on the eruption rates and lengths of the other incisors in the rat. Archives of Oral Biology 40: 467–471

Burn-Murdoch R A 1999 The length and eruption rates of incisor teeth in rats after one or more of them had been unimpeded. European Journal of Orthodontics 21: 49–56

Chiba M, Yamaguchi S, Komatsu K 1996 Measurement of the force needed to restrain eruptive movement of the rat mandibular incisor. Archives of Oral Biology 41: 341–349

de Carlos F, Cobo J, Diaz-Esnal B, Arguelles J, Vijande M, Costales M 2006 Orthodontic tooth movement after inhibition of cyclooxygenase-2. American Journal of Orthodontics and Dentofacial Orthopedics 129: 402–406

Drevenšek M, Sprogar Š, Boras I, Drevenšek G 2006 Effects of endothelin antagonist tezosentan on orthodontic tooth movement in rats. American Journal of Orthodontics and Dentofacial Orthopedics 129: 555–558

Guajardo G *et al.* 2000 Immunohistochemical localization of epidermal growth factor in cat paradental tissues during tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 118: 210–219

Harari D, Hermolin G, Harari O 2005 The effect of age on morphology and eruption of the lower incisors in mature rats. Archives of Oral Biology 50: 953–958

Jäger A *et al*. 2005 Soluble cytokine receptor treatment in experimental orthodontic tooth movement in the rat. European Journal of Orthodontics 27: 1–11

Law K T, Lee C K, King N M, Rabie A B 2003 The relationship between eruption and length of mandibular incisors in young rats. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research 9: 47–53

Lee C K, Law K T, King N M, Rabie A B 2002 A comparison between a conventional optical method and image-analysis for measuring the unimpeded eruption rate of the rat mandibular incisor. Archives of Oral Biology 47: 555–562

Mermut S, Bengi O, Akin E, Kürkçü M, Karaçay S 2007 Effects of interferon-gamma on bone remodeling during experimental tooth movement. Angle Orthodontist 77: 135–141

Moxham B J 2002 The effects of aging on the eruptive behavior of the mandibular incisor in male rats. Connective Tissue Research 43: 280–282

Ong C K, Walsh L J, Harbrow D, Taverne A A, Symons A L 2000 Orthodontic tooth movement in the prednisolone-treated rat. Angle Orthodontist 70: 118–125

Pillon J J, Kuijpers-Jagtman A M, Maltha J C 1996 Magnitude of orthodontic forces and rate of bodily tooth movement. An experimental study. American Journal of Orthodontics and Dentofacial Orthopedics 110: 16–23

Ren Y, Maltha J C, Kuijpers-Jagtman A M 2004 The rat as a model for orthodontic tooth movement—a critical review and a proposed solution. European Journal of Orthodontics 26: 483–490

Risnes S, Septier D, Goldberg M 1995 Accelerated eruption of rat lower incisor. Relationship between impeded and unimpeded eruption rates, rate of attrition, tooth length, and production of dentin and enamel. Connective Tissue Research 32: 183–189

Schour I, Massler M 1949 The teeth. In: Farris E J, Griffith J Q (eds). The rat in laboratory investigation. Lippincott Co., Philadelphia, pp. 104–160

Shimada A, Shibata T, Komatsu K, Chiba M 2003 The effects of intrusive loading on axial movements of impeded and unimpeded rat incisors: estimation of eruptive force. Archives of Oral Biology 48: 345–351

Shimada A, Shibata T, Komatsu K 2004 Relationship between the tooth eruption and regional blood flow in angiotensin II-induced hypertensive rats. Archives of Oral Biology 49: 427–433

van Leeuwen E J, Maltha J C, Kuijpers-Jagtman A M 1999 Tooth movement with light continuous and discontinuous forces in beagle dogs. European Journal of Oral Science 107: 468–474

Verna C, Dalstra M, Lee T C, Cattaneo P M, Melsen B 2004 Microcracks in the alveolar bone following orthodontic tooth movement: a morphological and morphometric study. European Journal of Orthodontics 26: 459–467

von Böhl M, Maltha J C, Von Den Hoff J W, Kuijpers-Jagtman A M 2004 Focal hyalinization during experimental tooth movement in beagle dogs. American Journal of Orthodontics and Dentofacial Orthopedics 125: 615–623

Weinreb M, Gal D, Weinreb M M, Pitaru S 1997 Changes in the shape and orientation of periodontal ligament fibroblasts in the continuously erupting rat incisor following removal of the occlusal load. Journal of Dental Research 76: 1660–1666

Yamaguchi S, Chiba M, Yamane A, Ohshima S, Komatsu K 1997 Dose-response effects of adrenergic drugs on axial movements of the rat mandibular incisor and on arterial blood pressure. Archives of Oral Biology 42: 801–809