European Journal of Orthodontics 29 (2007) 605–608
doi:10.1093/ejo/cjm064
Advance Access publication 18 September 2007

The effects of TBC3214, a selective endothelin ET_A receptor antagonist, on orthodontic tooth movement in rats

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SUMMARY Many chemical messengers are involved in the process of alveolar bone and periodontal ligament remodelling during orthodontic tooth movement. Among them is probably endothelin-1 (ET-1). Its role in this process has been partly explained using tezosentan, which affects endothelin A (ET_A) and endothelin B (ET_B) receptors. Tezosentan enhances orthodontic tooth movement. The aim of this study was to determine the possible effects of a highly selective ET_A antagonist on orthodontic tooth movement in rats.

Thirty male Wistar rats, 11–12 weeks of age, divided into three equal groups. In group I, a closed-coil spring was used and they were treated daily with 15 mg/kg body weight of TBC3214, a highly selective ET_A antagonist. A closed-coil spring was also used in group II and the animals were treated daily with a placebo. Group III were treated daily with a placebo. The coil spring delivered a force of 25 cN and was attached between the upper left first molar and upper left incisor. The distance between the teeth was measured with a digital calliper (accuracy ± 0.01 mm) on days 0, 7, 14, 21, 24, 32, 37, and 40. The differences in the distance between the teeth were calculated to determine the amount of tooth movement. Statistical analysis was performed using two-way analysis of variance, Bonferroni's correction, and paired *t*-tests.

The distance between the upper left first molar and the upper left incisor decreased in groups I and II. In group I, tooth movement was significantly less on days 32 and 37 ($P<0.01$) and on day 40 ($P<0.001$) compared with group II. In group III, the distance between the teeth increased during the study ($P<0.001$). In animals treated daily with TBC3214, tooth movement was significantly less compared with the animals treated with a placebo. It is concluded that ET-1, which is the predominant form of endothelin isopeptides, is involved in orthodontic tooth movement in rats, probably by enhancing bone resorption via ET_A receptors.

Introduction

Remodelling of the periodontal ligament (PDL) and alveolar bone is one of the key processes of orthodontic tooth movement (Meikle, 2006). Many chemical messengers are involved in this process. Among them could be endothelin-1 (ET-1), which is released during intravascular shear stress, hypoxia, and ischaemia (Kourembanas *et al.*, 1991; Rubanyi and Polokoff, 1994; Schmitz-Spanke and Schipke, 2000). All these phenomena are also present during force application, so ET-1 could be released in the PDL and alveolar bone during orthodontic tooth movement. An upregulation of ET-1 expression in the PDL microvasculature following acute tooth loading in marmoset monkeys has already been established (Sims *et al.*, 2003).

The role of ET-1 in the process of orthodontic tooth movement has been partially explained using tezosentan, an endothelin A (ET_A) and endothelin B (ET_B) receptor antagonist. Tezosentan enhanced orthodontic tooth movement in rats after 25 days of treatment (Drevenšek *et al.*, 2006).

The endothelin system consists of four active endothelin isopeptides (ET-1–ET-4), and three specific endothelin receptors (ET_A, ET_B, and ET_C). ET-1 is the predominant and most important isoform of endothelin in humans (Miyauchi and Masaki, 1999; Rich and McLaughlin, 2003). It has been established that it is produced by endothelial cells, fibroblasts, smooth muscle cells, leukocytes, macrophages, epithelial cells, and osteoblasts (Matsumura *et al.*, 1989; Ehrenreich *et al.*, 1990; Ohta *et al.*, 1990; Scott-Burden *et al.*, 1991; Sessa *et al.*, 1991; Zeballos *et al.*, 1991; Cybulsky *et al.*, 1993; Levin, 1995).

Both ET_A and ET_B receptors have been found on osteoblasts (Stern *et al.*, 1995; Kasperk *et al.*, 1997). ET-1 stimulates their proliferation (Kasperk *et al.*, 1997; von Schroeder *et al.*, 2003) and differentiation and activity (Nelson *et al.*, 1999; von Schroeder *et al.*, 2003; Guise and Mohammad, 2004). ET-1 also stimulates the formation of extracellular bone matrix proteins (Stern *et al.*, 1995) and bone (Tatrai *et al.*, 1992), but inhibits osteoblast mineralization via ET_A receptors (Hiruma *et al.*, 1998). Long-term inhibition of bone ET_A receptors causes less bone formation and osteopenia in growing rats (Tsukahara *et al.*, 1998). While it appears that ET-1 stimulates bone formation predominantly via ET_A receptors on osteoblasts, the mechanism of ET-1 acting on endothelin receptors on osteoclasts is unknown.

The influence of ET-1 on bone resorption is controversial. ET-1 decreases bone resorption by inhibition of osteoclast motility (Alam *et al.*, 1992; Nelson *et al.*, 1999). A study performed on prostate cancer cells showed that osteoclastic bone resorption was blocked by the presence of ET-1 in a dose-dependent manner (Chiao *et al.*, 2000). On the other hand, it was established that ET-1 stimulates prostaglandin-dependent bone resorption (Tatrai *et al.*, 1992; Stern *et al.*, 1995). The endothelin antagonist, tezosentan, which acts on ET_A and on ET_B receptors, has been shown to increase tooth movement (Drevenšek *et al.*, 2006).

The aim of the present study was to determine the involvement of ET_A receptors in orthodontic tooth movement. Tooth movement modulated by applying an endothelin antagonist, acting only on ET_A receptors, could help in understanding the role of ET-1 in the process of bone remodelling. A highly selective ET_A receptor antagonist, TBC3214, was used in this study.

Materials and methods

Animals

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

The study was carried out on 30 male Wistar rats (300–340 g, 11–12 weeks old). The animals were housed in groups of five in polycarbonate cages (Ehret, Emmendingen, Germany) under normal laboratory conditions [constant temperature (24–25°C) and humidity] with a 12-hour circadian cycle and fed with a diet of soaked standard laboratory rat chow (KZ Krka d.o.o., PE Krmila, Novo mesto, Slovenia) and water *ad libitum*. The rat chow was soaked in water to facilitate food intake.

Chemicals

A mixture of three anaesthetics was used to ensure general anaesthesia under which the placement of the closed-coil spring was performed. The anaesthetics were injected intraperitoneally: ketamine (Bioketan, Vetoquinol Zaklady Farmaceuticzne Biowet Gorozow, Poland, 50 mg/kg body weight), medetomidin hydrochloride (Domitor, Pfizer Animal Health, Louvain-la-Neuve, Belgium, 67 µg/kg body weight), and thiopental (Tiopental, Pliva, Zagreb, Croatia, 3 mg/kg body weight).

Orthodontic appliance

The orthodontic appliance consisted of a superelastic closed-coil spring (25 cN, wire diameter 0.15 mm, GAC International, Bohemia, New York, USA) placed between the first maxillary molar and the incisors. The closed-coil spring was attached to the upper left first molar with a stainless steel ligature wire (diameter 0.25 mm, Dentaurum, Ispringen, Germany) and to the incisors by surgical steel wire (4-0, multifilament, W310, Ethicon, Johnson & Johnson, New Jersey, USA). To improve fixation of the appliance, a 0.5-mm hole was made using a hard metal burr (HM 1, 204, 005, Meisinger, Neuss, Germany). The hole was drilled through the aproximal tooth surfaces, perpendicular to the longitudinal axis of the incisors at the gingival level. The steel wire was inserted through the hole and bent on the aproximal surface of the right incisor (Figure 1). Light curing bonding material (Tetric flow, Ivoclar Vivadent, Schaan, Lichtenstein) was used to protect the soft tissues from the sharp wire endings.

Study protocol

The study protocol was set to 6 weeks since it takes a few weeks before tooth movement reaches the linear phase and 'real' tooth movement through bone occurs.

The animals were divided into three groups ($n = 10$):

Group I: a closed-coil spring was used and the animals were treated daily, at approximately the same time of day for 40 days, with 15 mg/kg body weight of TBC3214 subcutaneously. TBC3214 is an orally available, highly selective, ET_A receptor antagonist (more than 100 000-fold selective, ET_A versus ET_B receptor) (Wu *et al.*, 2001).

Group II: a closed-coil spring was used and the animals were injected daily with 0.1 ml of placebo (saline) subcutaneously, at approximately at the same time of day for 40 days.

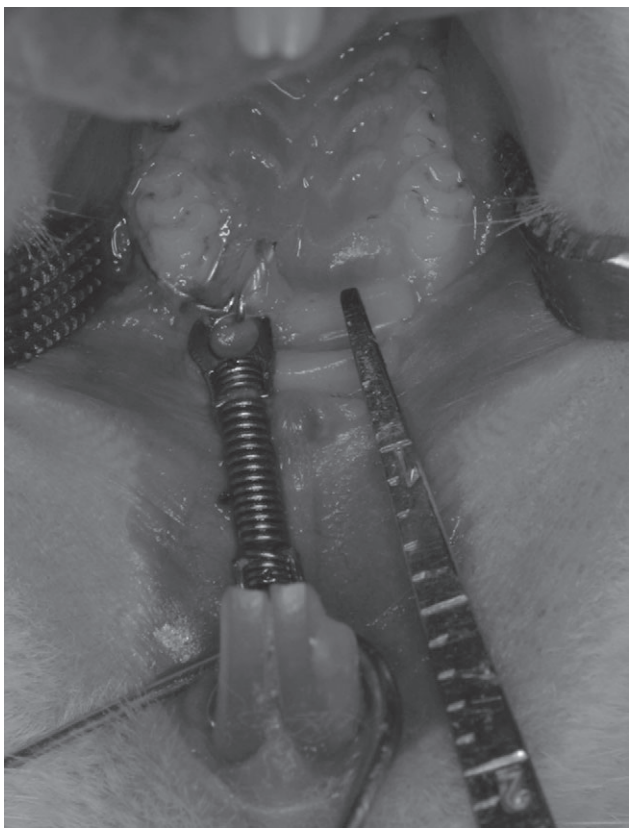

Figure 1 Photograph showing the position of the drilled hole and of the orthodontic appliance.

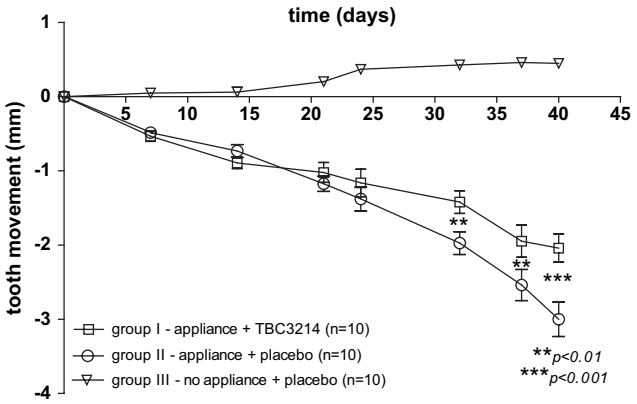

Figure 2 Tooth movement on the experimental side. Tooth movement was significantly less in group I compared with group II.

Group III: the animals were injected daily with 0.1 ml of placebo (saline) subcutaneously, at approximately at the same time of day for 40 days.

Measurements and statistics

The distance between the most mesial point of the maxillary first molar and the most distal point of the ipsilateral incisor at the gingival level was measured on the experimental side (Figure 2). The measurements were undertaken using a digitronic calliper with an accuracy ±0.01 mm (144-15D, Wilson & Wolpert, Utrecht, The Netherlands) on days 0, 7, 14, 21, 24, 32, 37, and 40, while the animals were anaesthetized. All measurements were carried out twice by two investigators (JV and ŠS) independently within a few minutes. Inter-examiner reliability was tested with the intraclass correlation coefficient (ICC) and a paired *t*-test was used to assess systematic bias.

In order to test the superelastic properties and delivered force of the closed-coil springs, the following method was used. Ten out of 20 closed-coil springs were randomly selected from eight boxes. The springs were activated from 1 to 20 mm and the reproducible force of 25 cN was determined over a range of 3–8 mm activation (Drevenšek *et al.*, 2006).

Statistical analysis was performed using two-way analysis of variance and Bonferroni's correction in GraphPad Prism 4.00 (GraphPad Software, San Diego, California, USA).

Results

Systematic bias, which showed a value of $P>0.87$, was tested using a paired *t*-test. The ICC was found to be 0.94±0.02. Since reliability was within the standards, the mean value of the four measurements was used for further statistical analysis.

At the experimental side, a significant decrease was found in the measured distances between the incisors and molars between group I and group II on days 32 and 37 ($P<0.01$) and on day 40 ($P<0.001$). Changes in the distances significantly differed between groups I and II compared with group III on days 7, 14, 21, 24, 32, 37 and 40 ($P<0.001$; Figure 2). In the control group, the distances on the experimental side significantly increased ($P<0.001$) from day 0 to day 40.

Discussion

The results of the present study demonstrate that TBC3214, a selective ET_A antagonist, significantly reduces the rate of tooth movement in rats. A previous investigation has shown the possibility of ET-1 involvement in bone remodelling, using tezosentan, an ET_A/ET_B endothelin antagonist, which enhanced tooth movement in rats (Drevenšek *et al.*, 2006). Since tezosentan is an ET_A/ET_B endothelin antagonist, it could not be determined whether it enhanced tooth movement by acting on ET_A and/or on ET_B receptors.

Many studies have shown that ET-1 stimulates bone formation, acting predominantly on ET_A receptors (Tatrai *et al.*, 1992; Stern *et al.*, 1995; Kasperk *et al.*, 1997; Tsukahara *et al.*, 1998; Nelson *et al.*, 1999; von Schroeder *et al.*, 2003; Guise and Mohammad, 2004). However, studies on the influence of ET-1 on bone resorption are contradictory. Some have shown that ET-1 inhibits (Alam *et al.*, 1992; Nelson *et al.*, 1999; Chiao *et al.*, 2000), while others (Tatrai *et al.*, 1992; Stern *et al.*, 1995) have shown that ET-1 stimulates bone resorption. It is also not clear which endothelin receptor, if any, could be involved in bone resorption via ET-1. Tezosentan, a ET_A/ET_B receptor antagonist, was considered to increase bone resorption during the late phase of tooth movement (Drevenšek *et al.*, 2006).

Considering the existing data of the influence of ET-1 on bone resorption, two different explanations for the results of the present study can be postulated. On the one hand, it is possible that ET-1 enhances bone resorption via ET_A receptors. Consequently, an ET_A antagonist, such as TBC3214, would inhibit bone resorption and therefore decrease tooth movement in animals treated with ET_A antagonist, as shown in the present study. On the other hand, it is possible that ET-1 inhibits bone resorption indirectly. This hypothesis can be explained by the fact that during ischaemic and hypoxic conditions, which can be expected during orthodontic tooth movement, the number of ET_B receptors, which are responsible for endothelin clearance, increases (Kourembanas *et al.*, 1991). Consequently, a greater activity of ET_B versus ET_A receptors would result in less tooth movement, because of the increased endothelin clearance, which is mediated via the ET_B receptors.

There are several phases of orthodontic tooth movement. It takes from a few days to a few weeks before tooth movement reaches the linear phase and real tooth movement through bone occurs (Ren *et al.*, 2004). As shown in Figure 2, there was a difference in tooth movement between groups I and II throughout the study period, but significant differences were only noted from day 32. This was also shown in a previous investigation (Drevenšek *et al.*, 2006), where a significant difference in tooth movement between

the experimental and the control group was found on day 25. According to these results, it appears that ET-1 influences the late phase of orthodontic tooth movement.

Conclusions

Tooth movement was significantly less in animals treated with TBC3214, a highly selective ET_A antagonist, compared with those treated with a placebo. Therefore, it is concluded that ET-1, which is the predominant isoform of endothelin in humans (Miyauchi and Masaki, 1999; Rich and McLaughlin, 2003), is involved in the mechanism of orthodontic tooth movement in rats. Further studies, including histological, immunohistochemical, and biomolecular techniques, will establish a more exact role for endothelin receptors in orthodontic tooth movement.

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Funding

Slovenian Ministry of Higher Education, Science, and Technology (P3-0067).

Acknowledgements

We wish to thank Tommy A. Brock of Biological Sciences Encysive Pharmaceuticals for generously providing the ET_A antagonist TBC3214 for this study.

References

Alam A S *et al.* 1992 Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. Endocrinology 130: 3617–3624

Chiao J W *et al.* 2000 Endothelin-1 from prostate cancer cells is enhanced by bone contact which blocks osteoclastic bone resorption. British Journal of Cancer 83: 360–365

Cybulsky A V, Stewart D J, Cybulsky M I 1993 Glomerular epithelial cells produce endothelin-1. Journal of the American Society of Nephrology 3: 1398–1404

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

Ehrenreich H *et al.* 1990 Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. Journal of Experimental Medicine 172: 1741–1748

Guise T A, Mohammad K S 2004 Endothelins in bone cancer metastases. Cancer Treatment and Research 118: 197–212

Hiruma Y *et al.* 1998 Endothelins inhibit the mineralization of osteoblastic MC3T3-E1 cells through the A-type endothelin receptor. American Journal of Physiology 275: R1099–R1105

Kasperk C H *et al.* 1997 Endothelin-1 is a potent regulator of human bone cell metabolism *in vitro*. Calcified Tissue International 60: 368–374

Kourembanas S, Marsden P A, McQuillan L P, Faller D V 1991 Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. Journal of Clinical Investigation 88: 1054–1057

Levin E R 1995 Endothelins. New England Journal of Medicine 333: 356–363

Matsumura Y, Ikegawa R, Ohyama T, Hayashi K, Morimoto S 1989 Endothelin immunoreactivity in medium from cultured porcine aortic endothelial cells correlates with the biological activity. Biochemical and Biophysical Research Communications 160: 602–628

Meikle M C 2006 The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. European Journal of Orthodontics 28: 221–240

Miyauchi T, Masaki T 1999 Pathophysiology of endothelin in the cardiovascular system. Annual Review of Physiology 61: 391–415

Nelson J B *et al.* 1999 New bone formation in an osteoblastic tumor model is increased by endothelin-1 overexpression and decreased by endothelin A receptor blockade. Urology 53: 1063–1069

Ohta K *et al.* 1990 Cytokine-induced release of endothelin-1 from porcine renal epithelial cell line. Biochemical and Biophysical Research Communications 169: 578–584

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

Rich S, McLaughlin V V 2003 Endothelin receptor blockers in cardiovascular disease. Circulation 108: 2184–2190

Rubanyi G M, Polokoff M A 1994 Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacological Reviews 46: 325–415

Schmitz-Spanke S, Schipke J D 2000 Potential role of endothelin-1 and endothelin antagonists in cardiovascular diseases. Basic Research in Cardiology 95: 290–298

Scott-Burden T, Resink T J, Hahn A W, Vanhoutte P M 1991 Induction of endothelin secretion by angiotensin II: effects on growth and synthetic activity of vascular smooth muscle cells. Journal of Cardiovascular Pharmacology 17: S96–S100

Sessa W C, Kaw S, Hecker M, Vane J R 1991 The biosynthesis of endothelin-1 by human polymorphonuclear leukocytes. Biochemical and Biophysical Research Communications 174: 613–618

Sims M R, Ashworth J F, Sampson W J 2003 Upregulation of immunoreactivity of endothelin-1 and α-SMA in PDL microvasculature following acute tooth loading: an immunohistochemical study in the marmoset. Orthodontics & Craniofacial Research 6: 74–82

Stern P H *et al.* 1995 Endothelin receptors, second messengers, and actions in bone. Journal of Nutrition 125: 2028S–2032S

Tatrai A, Foster S, Lakatos P, Shankar G, Stern P H 1992 Endothelin-1 actions on resorption, collagen and noncollagen protein synthesis, and phosphatidylinositol turnover in bone organ cultures. Endocrinology 131: 603–607

Tsukahara H, Hori C, Hiraoka M, Yamamoto K, Ishii Y, Mayumi M 1998 Endothelin subtype A receptor antagonist induces osteopenia in growing rats. Metabolism Clinical and Experimental 47: 1403–1407

von Schroeder H P, Veillette C J, Payandeh J, Qureshi A, Heersche J N 2003 Endothelin-1 promotes osteoprogenitor proliferation and differentiation in fetal rat calvarial cell cultures. Bone 33: 673–684

Wu C *et al.* 2001 Acyl substitution at the ortho position of anilides enhances oral bioavailability of thiophene sulfonamides: TBC3214, an ETA selective endothelin antagonist. Journal of Medicinal Chemistry 44: 1211–1216

Zeballos G A, An S J, Wu J M 1991 Endothelin-1 secretion by human fibroblasts in culture: effects of cell density and IFN-beta. Biochemistry International 25: 845–852