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Strontium ranelate improved tooth anchorage and reduced root resorption in orthodontic treatment of rats

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ABSTRACT

The anchorage mechanisms currently used in orthodontic treatment have various disadvantages. The objective of this study was to determine the applicability of the osteoporosis medication strontium ranelate in pharmacologically induced orthodontic tooth anchorage.

In 48 male Wistar rats, a constant orthodontic force of 0.25 N was reciprocally applied to the upper first molar and the incisors by means of a Sentalloy® closed coil spring for two to four weeks. 50% of the animals received strontium ranelate at a daily oral dosage of 900 mg per kilogramme of body weight. Bioavailability was determined by blood analyses. The extent of tooth movement was measured both optometrically and cephalometrically (CBCT). Relative alveolar gene expression of osteoclastic markers and OPG-RANKL was assessed by qRT-PCR and root resorption area and osteoclastic activity were determined in TRAP-stained histologic sections of the alveolar process.

Compared to controls, the animals treated with strontium ranelate showed up to 40% less tooth movement after four weeks of orthodontic treatment. Gene expression and histologic analyses showed significantly less osteoclastic activity and a significantly smaller root resorption area. Blood analyses confirmed sufficient bioavailability of strontium ranelate.

Because of its pharmacologic effects on bone metabolism, strontium ranelate significantly reduced tooth movement and root resorption in orthodontic treatment of rats.

Strontium ranelate may be a viable agent for inducing tooth anchorage and reducing undesired root resorption in orthodontic treatment. Patients under medication of strontium ranelate have to expect prolonged orthodontic treatment times.

1. Introduction

Maintaining tooth anchorage during orthodontic treatment has challenged orthodontists since the beginning of orthodontic treatment. An important principle in orthodontic biomechanics is the third axiom of Newton: action = reaction (Graber, 2005). According to this principle, intentionally applied orthodontic forces exert unwanted contrary forces of equal size upon teeth used for anchorage purposes, resulting in their unwanted movement (Williams, 1995). Conventional methods for improving tooth anchorage aim at redirecting such forces to skeletal structures or distributing them over a larger number of teeth, thus reducing the effect on individual anchors. These methods include intramaxilliary and intermaxilliary intraoral anchorage systems, extraoral systems, such as headgear, and relatively modern approaches using palate bone implants for anchorage (Cousley, 2013; Proffit et al., 2007). Such implants have been effective in reducing the problem of patient compliance and avoiding anchorage loss by the direct redirection of forces into the bone. However, major drawbacks of this technique are high costs, the invasiveness of the procedure, which bears the risk of damaging nerves and other adjacent structures, and the necessity of surgical explantation after the end of therapy (Keles et al., 2007).

A relatively new and completely different approach to this problem is to employ pharmacologic modulators of bone metabolism that may reduce the need for complex mechanics and retention (Adachi et al., 1994). The effect of many such inhibitors on orthodontic tooth movement, the most promising being bisphosphonates, has been studied in animal models (Fujimura et al., 2009; Igarashi et al., 1994). However, numerous reports on

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bisphosphonate-associated necrosis of the jaw (BRONJ) after long-term treatment (Brozoski et al., 2012; Ghoneima et al., 2010) raise serious doubts about the future application in pharmacologic anchorage or retention, particularly because these side effects exclusively occur in the jaw.

As an alternative, strontium ranelate has been approved by the European Medicines Agency (EMA) for clinical use in October 2004. In contrast to previous drugs used for treating osteoporosis, strontium ranelate shows both anti-catabolic and anabolic properties in bone metabolism (Bonnelye et al., 2008; Neuprez et al., 2008), thus increasing bone strength and mass (Ammann et al., 2004; Marie, 2006) as well as reducing the risk of vertebral fractures (Reginster et al., 2005). In previous in vitro studies, we found a stimulating effect of strontium on osteoblasts of patients with *Dysostosis cleidocranialis* (Roemer et al., 2011) and on cells taken from human periodontal ligament (Roemer et al., 2012). Strontium ranelate may be a promising agent for pharmacologically induced orthodontic tooth anchorage, since known side effects, such as an increased risk of myocardial infarction (European Medicines Agency, 2013), could presumably be avoided by a topical administration route, since contrary to bisphosphonates they do not manifest themselves within the alveolar bone itself.

Although strontium ranelate has been investigated extensively for the treatment of osteoporosis, its possible application for or side effects on orthodontic treatment have not been examined so far. In the present study we evaluated the hypothesis, whether strontium ranelate affects the velocity and extent of orthodontic tooth movement.

2. Material and methods

2.1. Experimental design

For this study, we used 48 outbred male Wistar-rats (*Rattus norvegicus Berkenhout*) from the Charles River Laboratories (Sulzfeld, Germany, Crl:WI). In two subsequent experiments of 24 animals each, the rats underwent orthodontic treatment for two and four weeks respectively. For each experiment, the 24 animals were randomly divided into two groups of 12 animals each and either received strontium ranelate or physiological saline solution on a daily basis. The animals of each group were then randomly allocated into cages of four. To manage the daily workload, a blocking design was necessary using four animals (one cage) as the smallest experimental unit. Thus each of the two experiments was started with a time lag on six consecutive days, reducing orthodontic treatment and radiologic imaging to four animals per day.

The animal experiments were conducted with the approval and permission of the government of Upper Palatinate, Bavaria, Germany (approval ID: 54-2532.1-24/11) in accordance with the German Animal Welfare Act under supervision by an approved animal welfare officer.

The animals were kept in a conventional open-system S1 animal laboratory at the University of Regensburg (Germany). The laboratory had a temperature of 21 °C (*S.D.* 1 °C), a relative humidity of 55% (*S.D.* 10%) and a circulation of 16 air changes per hour at 25 Pa overpressure in a noise-free environment. The rats were only exposed to artificial fluorescent lighting in 12 h cycles of alternating light and dark periods (light phase: 7:00 a.m.–19:00 p.m.). The microbiologic status was protected according to the FELASA guidelines as described by Nicklas et al. (2002). The rats were kept in transparent type IV-polycarbonate cages (Makrolon®, 59.5 cm × 38 cm × 20 cm) with metal grid tops in an animal holding cabinet. The animals were bedded on standard germ-reduced ¾ fibre soft wood shavings (ssniff Spezialdiaeten GmbH, Soest, Germany), which were changed once a week. The rats were sustained with physiological saline solution from plastic bottles, which was changed twice a week, and a standard rat and mouse maintenance diet (V1535, ssniff, Soest, Germany). Water and chow were provided ad libitum except from 8:00 a.m. to 14:00 p.m., when chow was withheld. At the beginning of the orthodontic treatment, the solid chow pellets were mixed with physiological saline solution to make a soft mash, which was placed into a tray within the cage to prevent overstressing the orthodontic appliance during mastication.

Between shipment and the beginning of the experiment, the animals had an acclimatisation period of 10 days, resulting in a mean starting age of 40 days (*S.D.* 3 days) and a mean starting weight of 196 g (*S.D.* 30 g).

2.2. Pharmacologic treatment and monitoring

Pharmacologic treatment and weight monitoring started three weeks prior to orthodontic treatment and were conducted on a daily basis between 10:00 a.m. and 12:00 a.m. throughout the two experiments. The treatment groups received strontium ranelate at a dosage of 900 mg per kilogramme of body weight suspended in 1.5 ml of physiological saline solution by gavage feeding (Jones, 2012). Animals in the control groups only received 1.5 ml of physiological saline solution by the same means. The strontium ranelate source used (Protelos®, Servier Germany GmbH) contained approximately 50% maltodextrine, aspartame and mannitol (European Medicines Agency, 2012), thus 2.2 times (determined empirically) the calculated body-weight-dependent dose of strontium ranelate was used for the Protelos® dosage. We used two different probes for the treatment and the control groups to avoid inadvertent administration of traces of strontium ranelate to the control groups. Among the animals, the probes were disinfected by consecutive rinsing with 96% 2-propanol-ethanol and distilled water.

2.3. Orthodontic treatment

Orthodontic appliances were inserted after three weeks of medication as described by Kirschneck et al. (2013). Appliances consisted of a modified Sentalloy® closed coil spring (0.25 N, GAC International, Gräfelfing, Germany, 10-000-26) attached to the cervix of the upper first molar (on the animal's left side) that was then stretched to and fixated at the base of the upper incisors (Fig. 1A). This procedure resulted in reciprocal movement of both anchorage locations towards each other.

2.4. Cone-beam computed tomography (CBCT)

At one-week interval starting from the day of appliance insertion, three-dimensional radiologic images of the animals' skull were taken by means of cone-beam computed tomography (CBCT) as described by Kirschneck et al. (2013). During the experiment (Fig. 1B), we measured mesial tipping of the upper first molar and distalisation of the upper incisors as well as cranial growth in a defined two-dimensional sagittal *X*-plane at the treated jaw side. The obtained values were corrected for confounding variables (Kirschneck et al., 2013).

2.5. Blood sampling and processing

Blood was sampled to assess sufficient bioavailability and the systemic effect of the drug. We obtained blood samples of 1.5 ml from the retrobulbar venous plexus to determine the concentration of strontium ions directly in the blood as well as the activity of alkaline phosphatase as a marker of osteoblast activity (Bonnelye et al., 2008) according to the technique described by Stone (1954). Blood samples were obtained at the beginning of orthodontic treatment (after three weeks of medication) and repeated after two and four weeks. Alkaline phosphatase activity was determined at a precision of 1 U/l from serum samples using an enzymatic assay