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Reproductive toxicity of denosumab in cynomolgus monkeys

Jeanine L. Bussiere[a,*], Ian Pyrah[a], Rogely Boyce[a], Dan Branstetter[b], Mark Loomis[a], Dina Andrews-Cleavenger[a], Cynthia Farman[c,1], Glenn Elliott[c], Gary Chellman[c]

[a] Amgen Inc., One Amgen Center Dr., Thousand Oaks, CA 91320, United States
[b] Amgen Inc., 1201 Amgen Court West, Seattle, WA 98119, United States
[c] Charles River PCS-NV, 6995 Longley Lane, Reno, NV 89511, United States

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ABSTRACT

Denosumab is a monoclonal antibody that inhibits bone resorption by targeting RANKL, an essential mediator of osteoclast formation, function, and survival. Reproductive toxicity of denosumab was assessed in cynomolgus monkeys in an embryofetal development study (dosing GD20–50) and a pre-postnatal toxicity study (dosing GD20–parturition). In the embryofetal toxicity study, denosumab did not elicit maternal toxicity, fetal harm or teratogenicity. In the pre-postnatal toxicity study, there were increased stillbirths, and one maternal death due to dystocia. There was no effect on maternal mammary gland histomorphology, lactation, or fetal growth. In infants exposed in utero, there was increased postnatal mortality, decreased body weight gain, and decreased growth/development. Denosumab-related effects in infants were present in bones and lymph nodes. There was full recovery at 6 months of age from most bone-related changes observed earlier postpartum. The effects observed in mothers and infants were consistent with the pharmacological action of denosumab.

1. Introduction

The RANK–RANKL (receptor activator of nuclear factor kappa-B ligand) system is an essential mediator of osteoclast formation, function, and survival [1]. RANKL binds RANK on osteoclast precursors and on mature osteoclasts to promote differentiation of the precursor cells into osteoclasts and to stimulate mature osteoclasts to resorb bone. Osteoprotegerin (OPG) is the naturally occurring soluble decoy receptor that binds to and blocks the action of RANKL leading to an increase in bone mass [2]. Therefore, inhibition of RANKL is a biologically plausible intervention point in diseases associated with increased bone resorption.

Although the role of RANK/RANKL is predominantly control of osteoclast formation, function and survival, RANK/RANKL are also expressed on cells of the immune system. For example, RANKL knockout mice have been reported to have decreased number and proliferation of epidermal Langerhans cells [3]. RANK/RANKL knockout mice also fail to develop lymph nodes, although Peyer's patches are still formed normally in the intestine and dendritic cell development and function are normal [4,5]. The presence of RANK and RANKL appear to be essential mediators for the differentiation of lymph node CD3−CD4+CD45+ 'inducer' cells during embryogenesis of the lymph node at approximately gestation day 14 in mice [6]. Altered development and differentiation of lymphocytes was also seen in the knock-out mice, and RANKL deficient mice had deficient early B cell development and reduced thymic cellularity [4,5]. In RANK–deficient mice, there was a reduction in splenic B cells which is thought to be a result of altered bone microenvironment secondary to osteopetrosis [7].

In contrast, inhibition of RANK–RANKL signaling by transgenic over-expression of OPG in mice or rats did not lead to defects in lymph node formation [8]. These observations suggest that the inhibition of RANKL–RANK signaling might permit the development of lymph nodes and a normal functioning immune system, while total genetic ablation of RANKL–RANK signaling does not. The amount of RANKL required for normal lymph node development in a fetus is not known, however OPG concentrations in the OPG-transgenic rat embryos were approximately 100-fold greater than normal rats on gestational day 11 [9], which is prior to lymph node formation [6]. This level of OPG over-expression increased bone mass but did not prevent the development of normal lymph nodes [10].

RANKL also plays a role in the development of the mammary gland in the mouse, as RANK/RANKL knock-out mice exhibit absence of lactation through inhibition of mammary gland maturation [11,12]. The failure of lobulo-alveolar gland development during pregnancy led to the inability of dams to sufficiently

* Corresponding author at: Amgen Inc. One Amgen Center Dr., MS 29-2-A, Thousand Oaks, CA 91320-1799, United States. Tel.: +1 805 447 6182; fax: +1 805 499 2936.
E-mail address: jeanine.bussiere@amgen.com (J.L. Bussiere).
[1] Current address: Genentech Inc., One DNA Way, South San Francisco, CA 94080, United States.

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nurse their offspring. RANK–RANKL regulates mammary epithelial cell proliferation during lobulo-alveolar morphogenesis in an autocrine/paracrine fashion via spatially and temporally restricted expression patterns in the developing gland and the regulation of RANKL expression by progesterone (reviewed by Schramek and Penninger) [13].

Absence of osteoclasts and bone resorption in RANK/RANKL knockout mice during skeletal development results in osteopetrosis and failure of tooth eruption [14,15]. Similarly, partial inhibition of bone resorption in rats treated with a bisphosphonate resulted in delayed tooth eruption [16]. Molar tooth eruption in rats and humans is considered to share similar mechanisms [17]. Molar eruption is often inhibited in osteopetrotic humans with impaired osteoclast activity [18–20] and delayed tooth eruption has been reported in children with osteogenesis imperfecta treated with bisphosphonates [21].

Denosumab is a fully human IgG2 monoclonal antibody that inhibits bone resorption by targeting RANKL. Denosumab is approved in the U.S. under the tradename XGEVA® (denosumab 120 mg given every 4 weeks) for the prevention of skeletal-related events in patients with bone metastases from solid tumors [22]. Denosumab is also approved in the U.S. as Prolia® (denosumab 60 mg given every 6 months) for the treatment of postmenopausal women with osteoporosis at high risk for fracture; treatment to increase bone mass in men with osteoporosis at high risk for fracture; treatment to increase bone mass in men at high risk for fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer; and treatment to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer [23].

Denosumab binds to and neutralizes the activity of human and nonhuman primate RANKL but does not bind to rodent RANKL. Denosumab is highly immunogenic in monkeys and leads to the formation of binding and neutralizing anti-drug antibodies (ADA) necessitating higher doses in the chronic studies to allow evaluation of pharmacological activity. It is common for animals to develop antibodies toward a biopharmaceutical [24,25] as human proteins are seen as foreign by the animal's immune system.

Reproductive toxicity was assessed in cynomolgus monkeys to better understand the effects of denosumab. Treatment with denosumab had no effect on female fertility or male reproductive organs in monkeys at doses that were 13- to 50-fold higher than the recommended human dose of 60 mg subcutaneously administered once every 6 months, based on body weight (mg/kg) [22] or 6.5- to 25-fold based on 120 mg given every 4 weeks [23].

Adequate designs in nonhuman primates are available for developmental toxicity (embryofetal development, pre-postnatal development, enhanced pre-postnatal development), reproductive toxicity (male and female), and juvenile toxicity studies [26]. Therefore, an embryofetal development (EFD) study was conducted to assess potential embryonic and teratogenic effects of denosumab when administered subcutaneously (SC) to pregnant cynomolgus monkeys during the period of organogenesis (gestation days [GD] 20–50). This study was conducted early in the development program when this was the standard design for EFD studies in cynomolgus monkeys. However, because endogenous antibodies are known to cross the placenta predominantly during the fetal period in humans [27,28], and cynomolgus monkeys [29], current guidance is to consider dosing throughout the gestation period [30]. Moreover, the macaque lymphoid organ system and lymphoid cell anatomical compartmentalization show evidence of development starting in the second trimester (GD65) and an adult-like morphology by the early 3rd trimester which is similar to humans [31]. Extending dosing into the fetal period would increase fetal exposure and provide a more robust study, particularly with regard to the developing immune system [26]. Allowing for delivery of infants also provides support for potential pediatric indications. Therefore, a pre-postnatal development toxicity (PPND) study was conducted to assess the effects of denosumab when administered SC to pregnant cynomolgus monkeys throughout gestation (GD20 through parturition) on maternal toxicity and to evaluate the infants for external, skeletal and behavioral effects, as well as growth and development for up to 6 months of age.

2. Materials and methods

2.1. Test and control articles

Denosumab was formulated at 30 mg/mL for the EFD study (diluted to 2.5, 5 or 12.5 mg/mL and dosed at a volume of 1 mL/kg for the 2.5, 5 and 12.5 mg/kg dose groups respectively), and 60 mg/mL for the PPND study (dosed neat at a volume of 0.83 mL/kg for the 50 mg/kg dose group) in 10 mM Na acetate, 5% sorbitol, pH 5.2. The test article was diluted to the target concentration using an appropriate volume of vehicle control article. The vehicle control was the same formulation without the active ingredient. Formulations were prepared daily on each dosing day immediately prior to dosing.

2.2. Test system (animals and care)

2.2.1. EFD study

Sixty-four pregnant cynomolgus monkeys (*Macaca fascicularis*) of Chinese origin were assigned to the study ($n = 16$/group). The females were sexually mature, weighed from 2.5 to 4.4 kg on day 20 of gestation, and were at least 3 years old. Sexually mature male animals were used for mating purposes only. The animals were housed in a climate-controlled room with a minimum of 10-air changes/h. The temperature and relative humidity ranges were 19–25 °C and 30–70%, respectively. Artificial lighting was controlled automatically to give a cycle of 12 h light and 12 h dark. Each animal was offered twice daily a commercial pellet diet for primates (Ssniff P10, Soest, Germany). In addition, the animals received fresh fruit approximately twice weekly and one slice of bread once weekly. Tap water (local water supply, Münster, Germany) was provided ad libitum via an automatic watering system or bottles. Adult females were housed individually in stainless-steel cages. Housing and study conduct were in accordance with the German Animal Protection Act (approval no. G51/2002). Each cage was provided with tools for environmental enrichment, such as movable stainless steel mirrors, colored plastic tools, or colored plastic balls.

2.2.2. PPND study

Fifty eight pregnant cynomolgus monkeys (*Macaca fascicularis*) of Chinese origin were assigned to the study ($n = 29$/group). The females were sexually mature, weighed from 2.6 to 4.7 kg on day 20 of gestation and were at least 4–8 years old. Sexually mature male animals were used for mating purposes only. Adult females were housed individually or with their respective infant in stainless-steel cages. Primary enclosures were as specified in the United States Department of Agriculture (USDA) Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Resources [ILAR] publication, 1996). The animals were housed in a climate-controlled room with a minimum of 10-air changes/h. The temperature and relative humidity ranges were 18–29 °C and 30–70%, respectively. Artificial lighting was controlled automatically to give a cycle of 12 h light and 12 h dark. Purina Primate Diet No. 5048 was provided daily in amounts appropriate for the size and age of the animals. This diet was supplemented with fruit or vegetables 2–3 times weekly. Also, small bits of fruit, cereal, or other treats were occasionally given to the animals as part of the Testing Facility's environmental enrichment program. Municipality tap