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Mechanistic investigations on the etiology of Risperdal® Consta®-induced bone changes in female Wistar Hannover rats

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ABSTRACT

RISPERDAL® CONSTA® is a long-acting, intramuscular formulation of risperidone microspheres for the biweekly treatment of schizophrenia and other psychiatric disorders. In a 24-month carcinogenicity study male and female Wistar Hannover rats received RISPERDAL® CONSTA® by intramuscular injection at dosages of 5 or 40 mg/kg once every 2 weeks. Bone changes described as "osteodystrophy" were observed by routine microscopic examination at 40 mg/kg in the sternum of female rats after 12 months, and in the sternum and stifle joint of both male and female rats after 24 months of treatment, respectively. To investigate the etiology of these bone changes, a 12-month mechanistic study was conducted in female Wistar Hannover rats at dosages of 5, 20 and 40 mg/kg once every 2 weeks. In addition to routine parameters, this study included bone markers, hormone measurements, and peripheral quantitative computed tomography (pQCT) and dual-energy X-ray absorptiometry (DXA) bone density measurements. It revealed a treatment-related reduction in metaphyseal trabecular bone density of the femur and tibia at 20 and 40 mg/kg, which was evident in the tibia from Week 13 of treatment onwards. There was no convincing evidence for any of the modes of action known to underlie trabecular bone loss in rats including renal, nutritional, or hepatic osteodystrophy, estrogen deficiency, hyperthyroidism or glucocorticoid excess. It is hypothetized that prolonged hyperprolactinemia accompanied by an increase in parathyroid hormone-related protein (PTHrP) levels and a slight hypoestrogenic state could have caused the reduced trabecular bone density in RISPERDAL® CONSTA®-treated rats. The relevance of this finding in terms of human risk is unknown.

1. Introduction

RISPERDAL® CONSTA® is an extended release microspheres formulation of the atypical antipsychotic drug risperidone, which is used in psychiatry for the treatment of patients with schizophrenia, schizoaffective disorder or bipolar disorder (Möller, 2007; Fagiolini et al., 2010). The RISPERDAL® CONSTA® formulation is composed of risperidone drug substance micro-encapsulated in a polylactide-co-glycolide polymer. It is administered to patients by deep intramuscular injection at a recommended dose of 25 mg once every 2 weeks.

In a 24-month intramuscular carcinogenicity study with a 12-month interim sacrifice conducted in male and female Wistar Hannover rats, RISPERDAL® CONSTA® was administered by intramuscular injection at dosages of 0 (vehicle control), 5 or 40 mg/kg once every 2 weeks. Routine histology examinations revealed test article-related bone changes at 40 mg/kg designated as "osteodystrophy" (unpublished results). To address a query by the United States Food and Drug Administration (FDA) on these bone changes a 12-month mechanistic study was performed which is described in this paper.

In the intramuscular carcinogenicity study, serum calcium and inorganic phosphate levels were increased in female rats after 6 and

Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; BMC, bone mineral content; BMD, bone mineral density; BUN, blood urea nitrogen; CT-scan, computed tomography scan; DXA, dual-energy X-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; FSH, follicle stimulating hormone; GLP, Good Laboratory Practice; LH, luteinizing hormone; NAG, *N*-acetyl-D-glucosaminidase; OECD, Organisation for Economic Co-operation and Development; PRL, prolactin; pQCT, peripheral quantitative computed tomography; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; RIA, radioimmunoassay; T3, triiodothyronine; T4, thyroxin; TSH, thyroid stimulating hormone; FDA, Food and Drug Administration.

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12 months of treatment at 5 and 40 mg/kg, and in male rats after 12 months of dosing at 40 mg/kg. Throughout the study serum prolactin (PRL) levels were increased in the test article-treated animals due to the dopamine D_2 antagonistic action of risperidone (Bowden et al., 1992; Kapur et al., 2002). As expected (Freeman et al., 2000; Ben-Jonathan and Hnasko, 2001), after 12 and 24 months of treatment PRL-mediated tissue changes were found in the genital tract and mammary glands of both sexes including a trend toward pseudopregnancy in females. In the 40 mg/kg-dosed animals renal, thyroidal and parathyroidal changes were observed, particularly at the end of the 24-month dosing period, which may be important in explaining the etiology of the bone changes. Particularly in females a mild increase in non-neoplastic renal changes including pelvic mineralization was found. Whereas a reduction in focal and diffuse thyroid C-cell hyperplasia was observed in male and female rats, a marginal increase in parathyroid hyperplasia was noted in female rats.

The bone changes in the above study were predominantly present in the sternum of female rats after 12 months, and in the sternum and stifle joint of both male and female rats after 24 months of treatment, respectively. The effect dose of 40 mg/kg was associated with a plasma exposure twice the exposure in humans at the maximum recommended dose of 50 mg once every 2 weeks. The plasma exposure at the no-effect dose of 5 mg/kg was one third the maximum human exposure. There were no bone-related effects observed in a 12-month dog study with RISPERDAL® CONSTA® at dose levels up to 20 mg/kg once every 2 weeks where plasma exposures were up to 14 times the maximum human exposure. The relevance of the bone changes observed with RISPERDAL® CONSTA® in rats in terms of human risk is unknown.

To determine the etiology of bone changes with RISPERDAL® CONSTA® the current 12-month mechanistic study was carried out with female Wistar Hannover rats intramuscularly injected at dosages of 0 (vehicle control), 5, 20 or 40 mg/kg once every 2 weeks. The protocol included bone marker and hormone measurements in addition to bone scans, and was specifically designed to elucidate whether the bone changes could be caused by any of the modes of action known to underlie bone loss in rats such as renal (Moscovici et al., 1996) or hepatic osteodystrophy (Van der Merwe et al., 2003), and changes in serum 1,25-dihydroxy-vitamin D or hormone levels (Allain et al., 1995; Hulley et al., 2002; Kalu, 1991).

2. Materials and methods

2.1. Test materials

Vials with commercial formulation of RISPERDAL® CONSTA® were supplied by Janssen Pharmaceutica NV (Beerse, Belgium) and reconstituted with 1 mL of diluent to obtain a concentration of 50 mg/mL of risperidone in the final formulation. Placebo microspheres were reconstituted by adding the same amount of diluent to each vial of placebo microspheres. The concentration of risperidone in the RISPERDAL® CONSTA® depot formulation was 391 mg/g of microspheres.

2.2. Animals and experimental design

The 12-month mechanistic study was conducted at CIT (Evreux Cedex, France) in compliance with Animal Health regulations and Good Laboratory Practice (GLP) regulations issued by the Organization for Economic Co-operation and Development (OECD). A total of 232 female Wistar Hannover (Crl:WI [GLX/BRL/Han] IGSBR) rats from Charles River Laboratories (l'Arbresle, France) were allocated according to body weight and clinical condition to one vehicle control group (i.e., Group 1) and three dosage groups using computerized randomization. The latter groups received RISPERDAL® CONSTA® at dosages of 5, 20 and 40 mg/kg, respectively, once every 2 weeks (i.e., Groups 2, 3, and 4). Each group contained 58 animals and the mean weight of the dosage groups approximated the mean weight of the vehicle control group. Each group was divided into four subgroups dedicated to specific investigations (Table 1). Subgroups A, B and D each comprised 14 animals, while subgroup C comprised 16 animals. All animals were housed in suspended wire-meshed cages containing two rats of the same group and were maintained under conventional conditions (12 h/12 h light/dark cycle, 50 ± 20% relative humidity, and 22 ± 2 °C temperature) with free access to a standard rodent pelleted diet (Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water.

At initiation of treatment the animals were approximately 6 weeks of age and had a mean body weight of 161 g (range 136–186 g). RISPERDAL® CONSTA® was administered at a constant concentration and the administered volume was adjusted to achieve the required dose levels. Dose volumes used were 0.1 mL/kg/injection for Group 2, 0.4 mL/kg/injection for Group 3, and 0.8 mL/kg/injection for Groups 1 and 4, respectively. Plastic syringes with a 20 G needle were used for the injection procedure and before each injection, the injection site (i.e., the thigh crural muscle area) was clipped free from hair. Two injection sites were used in rotation (right leg muscle on Day 1, left leg muscle in Week 3, right leg muscle in Week 5, etc.). All animals were sacrificed in Week 53, i.e., approximately 2 weeks after the last injection.

2.3. Parameters

During the course of the experiment, all animals were checked daily for mortality and clinical signs, body weight and food consumption were recorded regularly. Throughout the study various additional investigations were performed for logistical reasons on animals of different subgroups as summarized in Table 1.

2.3.1. Blood biochemistry and urinalysis

Blood samples were taken from the orbital sinus of animals under light isoflurane anesthesia. Prior to sampling for routine blood biochemistry parameters and bone markers, and/or during urine collection, the animals were individually placed in metabolism cages and food was withdrawn for an overnight period of at least 14 h. When urinalysis was scheduled water was also withdrawn; at the time of food and water withdrawal, the animals received 20 mL/kg of filtered water by gavage. Routine blood biochemistry and urinalysis parameters, together with urinary *N*-acetyl-D-glucosaminidase (NAG) levels and urinary creatinine clearance, were determined in subgroups A in Weeks 4, 12, 26, 38 and 50.

2.3.2. Bone marker and hormone measurements

From animals in subgroups B and C of each treatment group, blood was collected in Weeks 4, 12, 26, 38 and 50 and in subgroups A and D samples were collected in Weeks 5, 13, 27, 39 and 51. The 1-week difference between blood sampling times was related to the routine blood biochemistry collection in subgroup A. The sampling for prolactin and corticosterone measurements in subgroup D was separated from the other subgroup collections in order to reduce stress experienced by the animals. In subgroups A and B urine was sampled in Weeks 4, 12, 26, 38 and 50. Bone markers and hormones were measured as summarized in Table 2. All hormone analyses were performed at the Ecole Nationale Vétérinaire de Lyon, Département des Sciences Biologiques, Marcy L'Etoile, France.

2.3.3. Bone scans

2.3.3.1. In vivo peripheral quantitative computed tomography (pQCT) measurements. In vivo pQCT measurements were performed on the left tibia of animals belonging to subgroups B, under isoflurane anesthesia, in Weeks 5, 13, 27, 39 and 51. The measurements were performed using a XCT Research SA+ densitometer (Stratec Medizintechnik, Pforzheim, Germany). Initially a scout view was performed to determine the desired position of the measurement and establish the reference line similarly positioned for all bones. Subsequently, a computed tomography scan (CT-scan) was performed. Two sections, 0.5 mm apart, were taken in the tibial metaphysis, and one section in the diaphysis. The total, trabecular and (sub)cortical areas, and densities, were calculated in the slices of the metaphysis. The outer and inner threshold settings used to distinguish the bone tissue from soft tissue, and the trabecular bone from the cortical bone, were 280 and 650 mg/cm^3, respectively. The total and cortical areas, and densities, were calculated in the slices of the diaphysis. The threshold to distinguish the cortical bone from soft tissue was 710 mg/cm^3.

2.3.3.2. Ex vivo dual-energy X-ray absorptiometry (DXA) measurements. At the end of the study DXA measurements were performed on the left femur, left tibia, and sternum of all animals belonging to subgroups B. Upon sacrifice of the animals after completion of the treatment period, the bones were placed under 2.5 cm of saline solution in a container and positioned for measurement on the scanner deck of a Discovery A QDR Series X-ray bone densitometer (Hologic France). Bone mineral content (BMC in g) and bone area (in cm^2) were measured. Bone mineral density (BMD in g/cm^2) was calculated as the BMC divided by the projected bone area. BMD was calculated for the whole bone (femur, tibia and sternum), metaphysis (femur and tibia), diaphysis (femur and tibia), proximal femur and distal tibia.

2.3.3.3. Ex vivo pQCT measurements. pQCT measurements were performed on the left femur and sternum of all animals belonging to subgroups B. Upon sacrifice of the animals after completion of the treatment period, the same procedure was followed as described in Section 2.3.3.1 using the XCT Research SA+ densitometer (Stratec Medizintechnik, Pforzheim, Germany). Two sections were taken in the femoral metaphysis and sternum, and one section in the femoral diaphysis. The total, trabecular and (sub)cortical areas, and densities, were calculated in the slices of the femoral metaphysis and sternum. The total and cortical areas, and densities, were calculated in the slices of the femoral diaphysis.