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# Use of barusiban in a novel study design for evaluation of tocolytic agents in pregnant and neonatal monkeys, including behavioural and immunological endpoints☆

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**Abstract**

The oxytocin receptor antagonist barusiban, currently being developed for treatment of preterm labour, was investigated in pregnant cynomolgus monkeys with a 9-month postnatal follow-up of their offspring. The nature of barusiban, its indication, and the potential exposure of pre- and postnatal infants entailed the design of a unique protocol to investigate all aspects of maternal and offspring well-being. Barusiban was administered to the mothers from gestation day 85 until delivery with daily subcutaneous dosages up to 2.5 mg/kg body weight/day. There were no test article-related effects seen in the mothers at any time during the study. The postnatal examination of offspring included routine toxicological parameters, as well as specialised investigation of the immune, cardiovascular, renal and central nervous systems, including a full behavioural assessment. A full pathology examination of offspring was performed at the end of the 9-month postnatal period. No adverse infant findings occurred.


*Keywords:* Oxytocin; Atosiban; Parturition; Preterm labour; Cynomolgus; Immunotoxicity; Juvenile toxicity; Barusiban

## 1. Introduction

Barusiban is a synthetic peptide oxytocin receptor antagonist that is being developed for the treatment of preterm labour. By blocking oxytocin receptors in the uterus, such antagonists can delay the onset of parturition [1]. The role of oxytocin in parturition remains controversial, but the tocolytic effect of oxytocin antagonists such as atosiban (trade name Tractocile®) has been demonstrated in several species, and will induce uterine quiescence in normal labour [2]. Preterm labour represents a significant unmet medical need [3]. Estimates of preterm birth range from 5% in developed countries other than the United States, to 12% in the United States, to 25% in developing countries [3–5]. Preterm delivery is associated with as much as 70% of perinatal deaths in some developed countries [6].

Development of a tocolytic for world wide registration requires extensive documentation of safety, and the development of barusiban has included a full programme of non-clinical safety studies, including general toxicology studies, as well as peri- and postnatal, cross-fostering and fetal toxicity studies in rats and rabbits. Recent guidelines have, however, highlighted the need to investigate potential postnatal sequelae following exposure in utero to pharmaceuticals. In particular, assessment of possible effects on the developing immune, central nervous, cardiovascular, renal and other systems has been stressed in several guidelines [7–9]. It has been shown that following subcutaneous administration to pregnant rabbits and marmosets, barusiban can cross the placenta and distribute in the fetus (Ferring, unpublished data). It was therefore necessary to design a study which would take into consideration all aspects of maternal and fetal exposure, as well as determining whether exposure in utero had any influence on postnatal development.

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Concern has been expressed about the possible effects of oxytocin receptor antagonists on behaviour and development of various organ systems [10], due to blocking the natural interactions of oxytocin within these systems. It is known that the oxytocin receptor is expressed in the developing brain of the rat [11], and the connection between behavioural function and oxytocin has been investigated with respect to social interaction [12–15] and anxiety [15,16]. Also, oxytocin has been shown to be involved in the induction of maternal behaviour [17,18], and an intracerebral injection of an oxytocin antagonist has been demonstrated to inhibit maternal behaviour after birth [19]. Further, the developing organ systems of offspring, especially the renal system, are considered to be influenced by oxytocin [20].

The aim of this article is to describe the design of a novel study protocol in non-human primates to determine the potential peri- and postnatal effects of exposure to a tocolytic in utero. The utility of this study design is demonstrated by the example of barusiban. The antagonist was administered from the mid-second through to third trimester of pregnancy in monkeys to mimic the longest potential human exposure.

## 2. Materials and methods

This study was performed in purpose-bred, sexually mature cynomolgus monkeys (*Macaca fascicularis*), ranging in age from 4.3 to 12 years. This species was chosen as it is similar to humans with regards to reproductive processes, placental structure, and the relative timing of offspring development. The animals were purchased from the Texas Primate Center, Alice, TX, USA and Scientific Resources International Ltd., Reno, NV, USA. The animals were given access ad libitum to tap water and appropriate amounts of a certified primate diet. On occasion, the animals were offered small bits of fruit and other treats. The study was conducted at Charles River Laboratories, Preclinical Services NV, USA.

The test article, barusiban, Lot No. PPL-FE4400001, is a cyclic heptapeptide oxytocin analogue consisting of an N-terminal residue which is deaminated and a C-terminal residue reduced to a β-aminoalcohol:

[Mpa$^{1}$, D-Trp$^{2}$, alle$^{4}$, Abu$^{6}$, MeOrn-ol$^{7}$]oxytocin(1–7)

Barusiban was synthesised by PolyPeptide Laboratories Inc., Torrance, CA, USA. It was formulated in a 25 mM acetate buffer and kept refrigerated between daily treatments. Samples of the dose formulations taken during the treatment part of the study were transferred to Ferring Pharmaceuticals A/S, Copenhagen, Denmark, where the identity, purity, content and stability of the compound were verified.

The study consisted of two phases (1 and 2), with designs as summarised in Table 1. Treatment of the phase 1 animals commenced January 2004 and treatment of the phase 2 animals commenced April 2004. In each phase, the animals were given single daily subcutaneous administrations of barusiban in a shaved area of the back from day 85 of gestation until delivery. In phase 1, three groups of five animals each were administered daily doses of 0 (control), 0.1 or 2.5 mg barusiban/kg body weight/day. In phase 2, four groups of 10 animals each were administered daily doses of 0 (control), 0.1, 0.5 or 2.5 mg barusiban/kg body weight/day. The highest dosage level of 2.5 mg/kg/day was chosen based on previous monkey toxicity studies in which minor local injection site effects were observed. The low dosage of 0.1 mg/kg/day was chosen to represent the pharmacologically effective dosage level, and the middle dosage was set at the geometric mean of the low and high dosages. Two animals were added to the control group to replace two pregnancy losses (one abortion on gestation 110 and one stillbirth on gestation day 155). Phase 1 of the two-phase design was used to determine the effect of barusiban up until the time of parturition. In previous cross-fostering studies with barusiban and atosiban [10] in rats, there was a failure of milk let-down because oxytocin activity was blocked. It was determined from phase 1 of this study that this was not a problem in monkeys, therefore phase 2 could continue to be dosed until parturition. If a pregnant animal reached gestation day 175, a Caesarean section was performed.

Blood samples for toxicokinetic analysis were collected on day 85 and day 140 of pregnancy. Plasma concentrations of barusiban were determined and toxicokinetic parameters were calculated using WinNonlin Professional v. 5.0 (Pharsight Corporation, Mountain View, CA, USA). At parturition, each infant was subjected to a detailed physical examination. Mothers and infants were weighed regularly during the study, and clinical signs and food consumption were recorded daily.

### 2.1. Test batteries

#### 2.1.1. Ultrasound monitoring of phase 1 and 2 animals

All pregnant animals were subjected to ultrasound examinations on GD20–25 (for determination of pregnancy), GD50 (end of major organogenesis), GD80 (predose) and GD140 (close to parturition). The general condition of the fetus was examined and the following parameters were measured: humerus length, femur length, biparietal diameter, occipitofrontal diameter, head circumference and abdominal circumference. Measurements were recorded as described by Tarantal and Hendrickx [21].

#### 2.1.2. Neurobehavioural examination

On the day of birth, the resting and elicited muscle tone of all phase 1 and 2 infants was scored for the following joints: hip, knee, toes, shoulder, elbow, fingers and neck. The infants were also evaluated for the presence of left/right and fore/hind limb asymmetry.

On birth days 1, 3, 7 and 14, all phase 2 infants were examined using a neurobehavioural test battery based on the Brazelton Neonatal Behaviour Assessment Scale. The following behaviours were evaluated: righting, palmar grasp, clasp-grasp, prone progression, lip smack orient, oral reflexes, eye reflexes, Moro reflex and negative geotaxis. In addition, responsiveness was scored during the test session to assess build-up during the approximately 20 min of handling required to conduct the evaluations.

During days 90–100 post partum, at the onset of infant independency, the phase 2 mothers and their infants were on five separate occasions transferred to a quiet room where the interaction between the two was video-taped for 20 min. During a 2-week period prior to video-recording, the animals had been transferred to the quiet room for adaptation purposes. The video-recordings were scored by experienced observers unaware of the treatment groups of the animals and the following were recorded: percent time together, total mother–infant departures/reunions (transactions), percent successful transactions, infant initiated transactions and synchronous activity. These end points were adapted from the Ainsworth test used in children, and were incorporated due to the potential of oxytocin to affect mother/infant interactions.

The tests used in this neurobehavioural assessment have previously been used in the standardised assessment of infant monkeys [22,23].

#### 2.1.3. Infant cardiovascular assessment

This test battery consisted of an assessment of heart rate and heart sounds on birth day 1 (all infants); heart rate, heart sounds and blood pressure during weeks 4, 8, 13, 26 and 39 post partum (phase 2 infants); electrocardiographic recordings during weeks 13 and 39 post partum (phase 2 infants); heart weight measurements and cardiac histopathology (part of *post mortem* procedures).

#### 2.1.4. Immune function battery

The immune system was assessed by:

(1) Determining populations of B- and T-lymphocytes, T-helper and T-suppressor lymphocytes, natural killer cells and monocytes using flow cytometry on birth day 4 (phase 1 infants) and during birth weeks 4, 8, 13, 26 and 39 (phase 2 infants).
(2) Determining immunoglobulins (total IgG, IgM, IgA) in selected phase 2 infants during birth weeks 13, 33 and 39.
(3) Immunising selected phase 2 offspring with keyhole limpet haemocyanin (KLH) in birth weeks 34 and 36, and determining anti-KLH IgG and anti-