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Safety study of Ciprofloxacin in newborn mice

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ABSTRACT

Ciprofloxacin, a broad-spectrum antimicrobial agent belonging to the fluoroquinolone family, is prescribed off-label in infants less than one year of age. Ciprofloxacin is included in the European Medicines Agency priority list of off-patent medicinal products requiring evaluation in neonates. This evaluation is undergoing within the TINN (Treat Infections in Neonates) FP7 EU project. As part of the TINN project, the present preclinical study was designed to assess the potential adverse effects of Ciprofloxacin on neurodevelopment, liver and joints in mice. Newborn mice received subcutaneous Ciprofloxacin at 10, 30 and 100 mg/kg/day from 2 to 12 postnatal days. Peak plasma levels of Ciprofloxacin were in the range of levels measured in human neonates. We examined vital functions *in vivo*, including cardiorespiratory parameters and temperature, psychomotor development, exploratory behavior, arthro-, nephro- and hepato-toxic effects. We found no effect of Ciprofloxacin at 10 and 30 mg/kg/day. In contrast, administration at 100 mg/kg/day delayed weight gain, impaired cardiorespiratory and psychomotor development, caused inflammatory infiltrates in the connective tissues surrounding the knee joint, and moderately increased extramedullary hematopoiesis. The present study pleads for careful watching of cardiorespiratory and motor development in neonates treated with Ciprofloxacin, in addition to the standard surveillance of arthrotoxicity.

1. Introduction

Ciprofloxacin is a synthetic broad-spectrum antimicrobial agent, belonging to the fluoroquinolone family, and considered as effective against a wide range of Gram-positive and Gram-negative organisms. Ciprofloxacin interferes with DNA function by inhibiting bacterial DNA gyrate and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair and recombination. Ciprofloxacin is the only fluoroquinolone to be included on the list of "Essential Medicines for Children" by WHO.[1] Ciprofloxacin was approved for use in children 1 through 17 years of age in 2004, but

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[1] http://www.who.int/medicines/publications/TRS958June2010.pdf. Accessed July 28 2014.

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not in neonates (Bradley et al., 2011). However, Ciprofloxacin may be prescribed "off-label" for the treatment of suspected or proven gram negative infections in neonates, especially if premature. In such context, data evaluating potential neurodevelopmental toxicity as well as joint and hepatic toxicity are missing (Bradley et al., 2011; Forsythe and Ernst, 2007; Kaguelidou et al., 2011; Sendzik et al., 2009).

In order to gather information on the possible short- and long-term adverse effects of Ciprofloxacin, the European Medicines Agency (EMA) has included Ciprofloxacin in its priority list of off-patent medicinal products for evaluation in the pediatric population.[2] The evaluation of Ciprofloxacin is currently ongoing by the TINN EU project (Treat Infections in Neonates[3]), in view of the submission of a Pediatric Use Marketing Authorization (PUMA) for Ciprofloxacin in neonates (Jacqz-Aigrain, 2011). The present preclinical study was conducted as part of the TINN project to assess the potential adverse effects of Ciprofloxacin on development in juvenile mice.

In order to assess the developmental effects of Ciprofloxacin, we exposed pre-weanling mice to Ciprofloxacin from 2 to 12 days of postnatal age (PND2 to PND12). Firstly, we examined the possible neurotoxic effects of Ciprofloxacin chronic administration on neurodevelopment with a special focus on breathing and psychomotor developments, which are both particularly critical in preterm infants. Then, we looked for effects of Ciprofloxacin on kidneys and liver development, considering previous reports that Ciprofloxacin caused a higher overall risk of hepatotoxicity (Alshammari et al., 2014). Finally, we examined whether Ciprofloxacin produced anarthrotoxic effect, as previously observed in weight bearing joints of juvenile animals and also in pediatric populations (Adefurin et al., 2011).

2. Material and methods

2.1. Test substance

Solutions (40 mL) of 1 mg/mL, 3 mg/mL and 10 mg/mL Ciprofloxacin and a control solution without Ciprofloxacin were prepared in sterile water for injection (5% glucose, Freeflex®, Fresenius Kabi, France) with Ciprofloxacin powder (Sigma–Aldrich, Germany), lactic acid (8, 24, 80 and 20 mg, respectively) and chloride acid (40 μL). The pH was adjusted to pH 5 with sodium hydroxide in all solutions, except the 10 mg/mL solution in which it was adjusted to pH 4. Afterwards, solutions were transferred into sterile vials through 0.2 μm syringe filters (Pall PharmAssure, Pall Medical, France). These solutions were prepared under a laminar airflow cabinet and controlled by the Pharmacy Department of Robert Debré Hospital in Paris.

2.2. Animals

Mouse pups ($N = 230$ for blood sampling, $N = 150$ for physiological and locomotion tests, and $N = 150$ for psychomotor tests and organs collection) were obtained from outbred Swiss female mice (Charles River Lab, France) housed at 20 °C ± 1 °C with a 12 h light/dark cycle and fed *ad libitum* (SAFE, France). All litters were born between 8 pm and 8 am. The first day following birth was denoted Postnatal Day 0 (PND0). Unless mentioned otherwise, litters were culled the day of birth to obtain 10 pups per litter, and housed individually in standard Plexiglas cages (Tecniplast, France). The

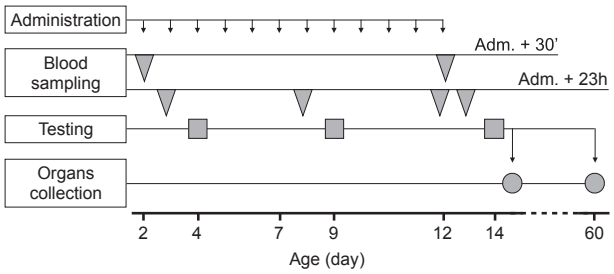

Fig. 1. Overview of our safety study of Ciprofloxacin in newborn mice. Arrows: All animals received a daily SC administration of Ciprofloxacin (0, 10, 30 or 100 mg/kg) from PND2 to PND12 (11 injections). Triangles: Animals (N = 230) were sacrificed to collect blood samples. Squares: Physiological and behavioral testing (N = 120), and psychomotor testing (N = 120) were carried out at PND4, PND9 and PND14. Circles: joints, kidneys and liver were collected at PND14 (N = 60) and PND60 (N = 56) to assess arthro-, nephro- and hepato-toxic effects of Ciprofloxacin.

animals were weighted daily from PND2 to PND14, and at PND16, PND21, PND30, PND41 and PND60. Animals were killed by decapitation at PND14 or by cervical dislocation at PND60. The overview of Ciprofloxacin safety testing in newborn mice is indicated in Fig. 1.

The present study complies with the EMA guidelines on the need for non-clinical testing in juvenile animals of pharmaceuticals for pediatric indications.[4] All the protocols were performed in accordance with the European Communities Council Directive (2010/63/UE) regarding the care and use of animals for experimental procedures, in compliance with the French regulations of the *Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale* (permission # A 94-028-21) and were approved by the Institutional Ethics Committee (Bichat-Robert-Debré) and national committees (Ministère de l'Enseignement Supérieur et de la Recherche – Direction Générale pour la Recherche et l'Innovation). All efforts were made to minimize animal suffering, especially by using non-invasive functional tests specially designed for newborn rodents.

2.3. Administration-route and doses

Ciprofloxacin was administered subcutaneously (SC). In newborn mice, intraperitoneal (IP) administration is poorly suited to chronic administration due to the risk of infection; oral administration is potentially harmful before PND12; intramuscular administration is difficult because of the paucity of muscle bulk; and intravenous (IV) administration is technically problematic because of the small size of blood vessels. We used a maximum dose of 100 mg/kg/day and intermediate doses of 10 and 30 mg/kg/day.

Ciprofloxacin solutions were administered once daily from PND2 to PND12 (11 injections per animal) at doses of 10 or 100 mg/kg/day in the blood sampling study, or at doses of 0, 10, 30 or 100 mg/kg/day in the physiological, psychomotor and behavioral studies. SC injections were alternatively performed at three different sites in the back of the mice, in order to minimize skin lesions.

2.4. Plasma levels

Blood samples were obtained from decapitation of three to five mice, pooled in an EDTA tube and centrifuged (4200 rpm at 4 °C) to obtain one plasma sample of 150 μL approximately and refrigerated

[2] EMEA/197972/2007; http://www.phytonetzwerk.de/Veranstaltungen/Kind_in_der_Apotheke/PNM_Sickmueller-EMEA_Stoffe_Off%20patent_May_2007en.pdf.

[3] http://www.tinn-project.org/.

[4] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003306.pdf.