Steroids 140 (2018) 1–10

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Dose response effects of postnatal hydrocortisone on growth and growth factors in the neonatal rat

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ARTICLE INFO

Keywords:
Hydrocortisone
Growth hormone
Insulin
Insulin-like growth factor-I
Leptin
Postnatal growth
Rats

ABSTRACT

Background and purpose: Hydrocortisone (HC), at different dosages, is used in critically ill newborns for lung stability, blood pressure support, and prevention of chronic lung disease (CLD). Its long-term effects on postnatal growth are not well studied. We hypothesized that early exposure to high doses of HC adversely affects growth, growth factors, metabolic hormones, and neurological outcomes, persisting in adulthood.

Experimental design: Rat pups received a single daily intramuscular dose of HC (1 mg/kg/day, 5 mg/kg/day, or 10 mg/kg/day on days 3, 4 & 5 postnatal age (P3, P4, P5). Age-matched controls received equivalent volume saline. Body weight, linear growth, and neurological outcomes were monitored. Animals were sacrificed at P21, P45, and P70 for blood glucose, insulin, IGF-I, GH, leptin, and corticosterone levels. Liver mRNA expression of IGFs and IGFBPs were determined at P21 and P70. Memory and learning abilities were tested using the Morris water maze test at P70.

Results: HC suppressed body weight and length at P12, P21 and P45, but by P70 there was catchup overgrowth in the 5 and 10 mg/kg/day groups. At P70 blood insulin, IGF-I, GH, and leptin levels were low, whereas blood glucose, and liver IGFs and IGFBPs were high in the high dose groups. High HC also caused delayed memory and learning abilities at P70.

Conclusions: These data demonstrate that while higher doses of HC may be required for hemodynamic stability and prevention of CLD, these doses may result in growth deficits, as well as neurological and metabolic sequelae in adulthood.

1. Introduction

Extremely low birth weight preterm infants with respiratory distress often show evidence of adrenal insufficiency during the first week of life [1].

Refractory hypotension is frequent in very low-birth weight infants, whose hypothalamic–pituitaryadrenal axis has been suggested to be immature [2]. In general, sicker and smaller infants have relatively low cortisol levels while in the neonatal intensive care unit [3]. Severe and prolonged hypotension is associated with increased mortality and central nervous system morbidity in critically ill preterm infants [4,5]. Attempts have been made to normalize blood pressure (BP), cardiac output (CO), and organ perfusion. In most hypotensive infants, cautious volume administration and the early use of low to medium dosages of

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https://doi.org/10.1016/j.steroids.2018.08.003
Received 3 July 2018; Received in revised form 8 August 2018; Accepted 13 August 2018
Available online 22 August 2018
0039-128X/

dopamine or epinephrine are effective in stabilizing the cardiovascular status and renal function. However, a subgroup of hypotensive preterm infants, do not respond to vasopressor inotropes. Critically ill preterm infants with vasopressor resistance are administered hydrocortisone (HC) to improve BP and renal function [6–9]. In these HC-treated infants, BP increases by 2 h after the first dose, and vasopressor inotrope requirement decreases by 6–12 h of treatment [7,9]. In addition to administering HC for physiologic deficiency, HC at different dosages is used in critically ill newborn infants for lung stability and prevention of chronic lung disease (CLD). While physiologic doses of 1 mg/kg may improve survival without bronchopulmonary dysplasia [10]; neurodevelopmental impairments at 2 years of age are not reduced [11–13]. Higher doses of 5 mg/kg appear to be more beneficial without adverse neurological outcomes [14–16], but may have detrimental effects on postnatal growth and cardiovascular outcomes in adult life.

There is a relative paucity of information regarding the effects of postnatal HC on anthropometric growth in preterm infants, compared to the numerous reports on brain volume and weight. A recent study by Tijsseling et al. [17] showed significant alterations in growth patterns for weight, height, and head circumference of preterm infants exposed to GCs. These growth impairments of HC, particularly in low birth weight for gestational age neonates may further predispose to metabolic syndrome in adulthood [18,19]. The mechanism(s) underlying the effects of early postnatal exposure to HC on postnatal growth may involve alterations in growth factors such as insulin-like growth factor (IGF)-I, insulin, and growth hormone (GH).

IGFs are pro-insulin-like polypeptides which markedly stimulate cell division and differentiation; and are major stimuli for pre- and postnatal growth [20]. The 3 peptide hormones in the IGF family are insulin, IGF-1 and IGF-2 and have 50% of their amino acids in common [21]. IGFs bind to IGF receptors (IGFR-1 and -2) and are modulated by six IGF-binding proteins (IGFBP-1 to -6) which are synthesized in the liver [22,23]. IGF deficiency has been shown to cause marked fetal growth retardation [24]. In the brain, IGF-1 is a potent inducer of oligodendrocyte development which is responsible for the synthesis of myelin [25,26]. Both IGF-I and IGF binding protein-3 (IGFBP-3) regulate early postnatal growth. Low serum IGF-I levels, measured in preterm infants, is associated with impaired growth, and development of retinopathy of prematurity as well as other complications [27–29]. IGF-I is produced in the liver, a major target for GH, which induces the production of IGF-I in the liver [30]. Insulin is a regulator of GH receptor and IGFBP-1, an endogenous inhibitor of IGF action. Serum cortisol is directly related to IGFBP-1 [31], suggesting that higher cortisol levels secondary to exogenous GC administration, may suppress IGF-1 and growth through induction of IGFBP-1. We therefore conducted a series of experiments to test the hypothesis that high doses of HC have lasting negative effects on the somatic growth, growth factors, metabolic hormones, and neurological outcomes which may persist into adulthood.

2. Materials and methods

2.1. Experimental design

All experiments were approved by the Institutional Animal Care and Use Committee, Long Beach Memorial Medical Center, Long Beach, CA. Animals were cared for according to the guidelines outlined by the Guide for the Care and Use of Laboratory Animals (National Research Council). Euthanasia of the animals was conducted according to the guidelines of the American Veterinary Medical Association (AVMA Panel). Certified infection-free timed-pregnant Sprague Dawley rats (200–300-gram body weight) were purchased from Charles River (Hollister, CA) and allowed to deliver spontaneously at term (22 days gestation). The pregnant rats (n = 12) were housed in individual cages and allowed to stabilize for 48 h under controlled environmental conditions with free access to food and water. Rat pups were pooled from 12 litters at P3 and were treated with a daily IM dose of 1 mg/kg/day HC (physiologic), 5 mg/kg/day (anti-inflammatory), or 10 mg/kg/day (stress) on P3, P4 & P5 (36 pups per treatment). A control group received equivalent volume sterile normal saline, IM. Total body weight and linear growth (nose to tail) were measured before treatment on P3, and post-treatment on P12, P21, P45, and P70. Neurological tests were performed before treatment, and at P12 and P21 post treatment. Food and water intake were determined from P21 (time of weaning from the dam) to P45 by gender. Food intake was determined by weighing the pellets using an electronic weighing balance, at baseline and at the end of each week. Water intake was determined by measuring the volume of water at baseline using a graduated cylinder, at baseline and at the same time each day. Pups were euthanized at P21, P45 and P70 (12 pups/treatment/age group). Prior to sacrifice at P70, rats were tested for memory and learning abilities using the Morris water maze test. At sacrifice, blood was collected for analysis of glucose, insulin, IGF-I, GH, leptin and corticosterone. Liver samples were analyzed at P21 and P70 for mRNA expression of IGF-I, IGF-II, IGFBP-1 and IGFBP-3. The brain, heart, lungs, liver, kidneys, pancreas, and spleen were removed and weighed.

2.2. Sample collection

At euthanasia, blood samples were collected for glucose levels using a glucometer. Blood samples were also collected for plasma and serum in appropriate tubes for insulin, IGF-I, GH, leptin, and corticosterone levels. For plasma samples, blood was collected in tubes containing EDTA and centrifuged at 3000 rpm for 20 min at 4 °C. The resulting plasma was aspirated and transferred to eppendorf tubes and frozen at −20 °C until assay. For serum samples, blood was collected in tubes containing no preservative and placed on ice to clot for 30 min. The samples were centrifuged at 3000 rpm for 20 min at 4 °C, and the resulting serum was aspirated and transferred to eppendorf tubes and frozen at −20 °C until assay. Animals were not fasted prior to blood sample collection for insulin and glucose measurements. Organ weights (brain, heart, lungs, liver, kidneys, spleen, and pancreas) were determined, and liver samples (100 mg) were collected, placed in tubes containing 1.0 mL TriZol reagent and snap frozen at −80 °C until analysis of IGF-I, IGF-II, IGFBP-1 and IGFBP-3 mRNA expression.

2.3. Assay of serum IGF-I, GH & leptin levels

Levels of IGF-1, GH, and letptin in serum samples were determined using commercially available enzyme immunoassay kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's protocol.

2.4. Assay of plasma insulin

Insulin levels in the plasma were determined using enzyme immunoassay kits purchased from Cayman Chemicals (Ann Arbor, MI, USA) according to the manufacturer's protocol.

2.5. Assay of plasma corticosterone

Plasma corticosterone levels were determined using enzyme immunoassay kits purchased from Enzo Life Sciences (Farmingdale, NY, USA) according to the manufacturer's protocol.

2.6. Isolation of total RNA

Total cellular RNA in the liver was extracted by homogenization using a polytron homogenizer (Brinkman Instruments, Inc., Westbury, N.J.). The homogenates were centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was collected and kept at room temperature for 5 min after which 200 µl chloroform per milliliter was added. The