Steroids 116 (2016) 76–82

Contents lists available at ScienceDirect

Steroids

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

Continuous corticosterone delivery via the drinking water or pellet implantation: A comparative study in mice

Sylvia J. Gasparini [a,*], Marie-Christin Weber [a,b], Holger Henneicke [a,c], Sarah Kim [a], Hong Zhou [a], Markus J. Seibel [a,d,*]

[a] Bone Research Program, ANZAC Research Institute, The University of Sydney, Sydney, Australia
[b] Department of Rheumatology and Clinical Immunology, Charité University Hospital, Berlin, Germany
[c] DFG-Center for Regenerative Therapies, Technische Universität Dresden, Dresden, Germany
[d] Concord Medical School, The University of Sydney, Sydney, Australia

ARTICLE INFO

Article history:
Received 10 October 2016
Accepted 26 October 2016
Available online 2 November 2016

Keywords:
Hypercortisolism
Corticosterone delivery
Glucocorticoid-induced dysmetabolism
Insulin-resistance
Sarcopenia
Osteoporosis

ABSTRACT

In order to investigate the effects of glucocorticoid excess in rodent models, reliable methods of continuous glucocorticoid delivery are essential. The current study compares two methods of corticosterone (CS) delivery in regards to their ability to induce typical adverse outcomes such as fat accrual, insulin resistance, sarcopenia and bone loss.

Eight-week-old mice received CS for 4 weeks either via the drinking water (25–100 μg CS/mL) or through weekly surgical implantation of slow release pellets containing 1.5 mg CS. Both methods induced abnormal fat mass accrual, inhibited lean mass accretion and bone expansion, suppressed serum osteocalcin levels and induced severe insulin resistance. There was a clear dose dependant relationship between the CS concentrations in the drinking water and the severity of the phenotype, with a concentration of 50 μg CS/mL drinking water most closely matching the metabolic changes induced by weekly pellet implantations. In contrast to pellets, however, delivery of CS via the drinking water resulted in a consistent diurnal exposure pattern, closely mimicking the kinetics of clinical glucocorticoid therapy. In addition, the method is safe, inexpensive, easily adjustable, non-invasive and avoids operative stress to the animals.

Our data demonstrate that delivery of CS via the drinking water has advantages over weekly implantations of slow-release pellets. A dose of 50 μg CS/mL drinking water is appropriate for the investigation of chronic glucocorticoid excess in mice.

1. Introduction

Glucocorticoid excess, whether endogenous or pharmacologically induced, is associated with a plethora of adverse outcomes; these include obesity, insulin resistance, sarcopenia and osteoporosis [1–8]. With an estimated 1–3% of the global adult population receiving long-term treatment with glucocorticoids [9], a better understanding of the mechanisms underlying the pathophysiology of hypercortisolism is paramount. However, while most of the research in this area has been performed using animal models, interpretation of results is often obfuscated by the wide variety of treatment delivery methods and dosages employed [4].

The effective delivery of exogenous corticosterone in mice is not a trivial issue. We previously demonstrated that two commonly employed methods of corticosterone (CS) delivery – daily injections and osmotic pumps – resulted in highly variable serum CS concentration and failed to reduce serum osteocalcin levels, thus were unreliable [10]. In contrast, delivery of CS via implantation of commercially available 'slow release pellets' was an effective method to induce hypercortisolism in mice. However, in contrast to the manufacturer's claims ("21-day release"), these pellets tend to discharge their contents within a few days, necessitating weekly pellet re-implantations. Hence, this method is not only expensive and time consuming, but also causes high levels of stress to the animals, potentially affecting experimental outcomes through the introduction of systematic errors in glucocorticoid hormone levels of control animals.

In the current study we therefore compared delivery of CS to mice via the drinking water as a non-invasive, stress-free tech-

* Corresponding authors at: Bone Research Program, ANZAC Research Institute, Sydney, NSW 2139, Australia (M.J. Seibel).
E-mail addresses: sylvia.gasparini@sydney.edu.au (S.J. Gasparini), markus.seibel@sydney.edu.au (M.J. Seibel).

http://dx.doi.org/10.1016/j.steroids.2016.10.008
0039-128X/

nique with the invasive method of pellet implantation [11]. As sarcopenia [1,6], obesity[7], insulin resistance [2,3] and osteoporosis [4,5] are all key features of chronic glucocorticoid excess, parameters reflecting these outcomes were assessed to determine whether delivery of CS via the drinking water could be used as an appropriate substitute for pellet implantation.

2. Experimental

2.1. Animal handling and blood collection

The study was performed in 8-week-old male CD1 Swiss white mice with a mean body weight (±S.D.) of 33.5 ± 2.7 g. All experiments were conducted with protocols approved by the institutional Animal Ethics Committee. Animals were maintained on a 12:12 h light–dark cycle (light cycle 06:00–18:00 h) with ad libitum access to standard chow and water. Day time blood collections were performed between 10:00–12:00 h and night time blood collections were performed between 22:00–24:00 h. Blood samples were obtained by non-terminal retro-orbital puncture, or by cardiac puncture at sacrifice. Both procedures were performed under general anaesthesia by intraperitoneal injection of Ketamine 0.075% and Xylazine 0.01%. Blood samples were allowed to clot at room temperature for 20 min followed by 15 min of centrifugation at 7500×*g* and 4 °C. Serum was collected and stored at −80 °C until further analysis. At sacrifice, the soleus muscle, the extensor digitorum longus (EDL) muscle and gonadal fat pads were harvested and weighed.

2.2. Corticosterone treatment

2.2.1. Implantation of slow-release pellets

Pellets containing 1.5 mg of CS (Innovative Research America, Sarasota, USA) were implanted subcutaneously into mice under general anaesthesia. Briefly, a small skin incision was made in the lower back and the pellet was placed under the skin. Using a trochar the pellet was then inserted distally from the entry point into the interscapular region and the incision was sutured. As our previous studies revealed that '21-day slow release pellets' were exhausted within 7 days of implantation [10,12], fresh pellets were reimplanted at weekly intervals. This treatment regimen corresponds to a dose of approximately 1.5 mg of CS/week. Mice were treated for four weeks.

2.2.2. Administration of corticosterone via the drinking water

Corticosterone powder (Sigma C2505) was first dissolved in 100% molecular grade ethanol and then added to the drinking water to a final concentration of 25, 50, 75 and 100 μg CS/mL and 1% ethanol. Placebo-treated groups received 1% ethanol in the drinking water. At an average water intake of 4.5 mL per mouse and day, the corresponding weekly dose of CS was calculated at 0.8, 1.6, 2.4 and 3.2 mg, respectively. Although no degradation of CS was detected over the course of a week (data not shown) the drinking water was replaced weekly with fresh corticosterone or ethanol. Mice were treated for four weeks.

2.3. Phenotypical assessment

2.3.1. Insulin tolerance tests

Insulin tolerance tests (ITT) were performed at the end of the four week treatment period. For ITTs, mice were fasted for 6 h prior to a baseline blood glucose reading. Insulin (Humalog, Eli Lilly and Company) was then injected intraperitoneally at a dose of 0.75 U/kg body weight. Blood glucose was measured at 0, 15, 30 and 60 min post-injection using an Accu-check glucometer (Roche). Blood glucose data is presented as percentage of baseline blood glucose ± SEM.

2.3.2. Body composition scans

Measurements of bone mineral content, bone area, lean mass and fat mass were performed on a Lunar PIXImus Densitometer for mice (GE Medical Systems) according to the manufacturer's instructions at baseline and at the end of the four week treatment period. Data is shown as percent change from baseline ± SEM.

2.3.3. Biochemical measurements

Plasma CS levels were measured by EIA (Arbor assays, Ann Arbor, USA) which has intra and interassay coefficients of variation of 6.3 and 7.5% respectively at a concentration of 2500 pg/mL and a limit of detection of 16.9 pg/mL. Serum total (i.e. carboxylated and uncarboxylated) mouse osteocalcin concentrations were determined by ELISA (Immutopics, San Clemente, USA) which has intra and interassay coefficients of variation of 3.7 and 6.1% respectively at a concentration of 25 ng/mL and a limit of detection of 0.4 ng/mL. Both assays were performed as per the manufacturer's instructions. Plasma corticosterone concentrations were measured 1, 3, and 7 days post pellet implantation. For the drinking water method CS was assessed at night time so as to capture the nocturnal increase in CS. Serum osteocalcin levels was measured in terminal serum samples.

2.4. Statistics

Group comparisons were performed using one-way ANOVA with Bonferroni multiple comparison post hoc test (more than two groups) or a Student's *t*-test (two groups), as appropriate. All calculations were performed using GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, USA).

3. Results

3.1. Circulating corticosterone levels

When CS was administered via the drinking water, night-time (active phase) plasma CS levels were found to be elevated in a dose dependent manner (Fig. 1A). In contrast, when blood was collected during the day time (sleep phase), plasma CS levels were lower in CS-treated than in vehicle-treated controls (data not shown). Being nocturnal animals, mice drink and thus ingest CS predominantly at night time, resulting in a typical pattern of diurnal CS variability as shown in Fig. 1B.

As in previous experiments [10,12] we measured significantly elevated serum CS levels only on the first day following pellet implantation. Despite the manufacturer's claims of slow release over 21 days, circulating CS levels returned to near-baseline by day 3, and to baseline levels by day 7 post implantation (Fig. 1C). Pellets were reimplanted weekly with similar kinetics [10].

3.2. Changes in body fat mass and insulin sensitivity

There was a clear dose dependent relationship between the amount of CS administered via the drinking water and whole body (Fig. 2A) and visceral (Fig. 2B) fat mass accrual. With a mean 40% increase in total body fat mass and an average gain of 450 mg in gonadal fat pad mass, a dose of 50 μg CS/mL drinking water produced changes in adipose tissue mass comparable to those achieved by pellet implantation. Mice on glucocorticoid treatment rapidly developed profound insulin resistance. With the exception of the lowest concentration (25 μg CS/mL), all CS doses administered via the drinking water induced complete insulin resistance