Journal of Pharmaceutical and Biomedical Analysis 149 (2018) 278–289

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Quantitative assessment of betamethasone dual-acting formulation in urine of patients with rheumatoid arthritis and ankylosing spondylitis after single-dose intramuscular administration and its application to long-term pharmacokinetic study

Arthur T. Kopylov[a,*], Alexander A. Novikov[b], Anna L. Kaysheva[a], Igor T. Vykhodets[c], Dmitry E. Karateev[b], Victor G. Zgoda[a], Andrey V. Lisitsa[a]

[a] V. N. Orekhovich Research Institute of Biomedical Chemistry, 10 Pogodinskaya str. bld. 8, 119121 Moscow, Russian Federation
[b] V. A. Nasonova Clinical Institute of Rheumatoid Arthritis, 34A Kashirskoe Highway, 115522 Moscow, Russian Federation
[c] Pirogov Russian National Research Medical University, 1 Ostrovitianov str., 117997 Moscow, Russian Federation

ARTICLE INFO

Article history:
Received 8 September 2017
Received in revised form 3 November 2017
Accepted 5 November 2017
Available online 6 November 2017

Keywords:
Glucocorticoids
Rheumatoid arthritis
Ankylosing spondylitis
Betamethasone
Long-term elimination
Combinatory therapy

ABSTRACT

Quantitative evaluation and assessment of pharmacokinetic parameters of Diprospan® (suspension for injection 7 mg/mL (2 mg + 5 mg/mL) of betamethasone) were performed in urine samples taken from patients with rheumatoid arthritis or ankylosing spondylitis for 28 days after systemic intramuscular administration in routine clinical practice in an open-comparative prospective cohort study. The maximum betamethasone concentration was reached at day 4 of the follow-up; in some cases, β-phase of elimination of the drug was appeared at day 14 or at day 21 of the follow-up. The deferred β-phase elimination was likely a consequence of the physiological characteristics of the patients or of the influence of non-steroidal agents. The half-life of betamethasone was 8.5 days. The elimination rate constant was 2.49 h-1; the mean clearance was 4.72 L/d. The recommended frequency of the drug administration to its complete elimination was estimated up to 48 days. Mann-Whitney test showed no significant differences in pharmacokinetic characteristics between male and female subjects. The prolonged elimination phase was observed in patients with deviations in their body mass index, continual treatment by diclofenac and nimesulide or, possibly, after consuming an alcohol. The study was recorded in Clinical Trials open source with identifier NCT03119454.

1. Introduction

Betamethasone is a synthetic fluorinated glucocorticoid with explicit anti-inflammatory properties, and as well as many other glucocorticoids are widely used in the treatment of rheumatoid arthritis (RA) and with ankylosing spondylitis (AS) [1–3]. High-efficiency discovery of glucocorticoids for treatment of RA and SA was a reason to honour Henchu, Kendal and Reichsten in 1950 by Nobel Prize in medicine. In patients with the early and low activity of RA insufficiency of the hypothalamic-pituitary-adrenal axis can be corrected by substituting therapy of glucocorticoids [4]. Guidelines of the American College of Rheumatology (2002) noted that "glucocorticoids in low doses (10 mg of prednisolone or less per day) are highly effective in relieving symptoms in patients with active RA" [5]. However, application of synthetic glucocorticoids may lead to undesirable side effects including risk of premature lethality [6], repression of antioxidant response by blocking NRF2-mediated cytoprotection [7], Cushing's syndrome [8] and bear a risk of fetal death in pregnancies [3]. Recently, in 2016 update, the European League Against Rheumatism (EULAR) recommended only a short-term usage of glucocorticoid, including betamethasone, as bringing therapy when initiating or changing with conventional synthetic disease-modifying anti-rheumatic drugs (DMARD) in patients with RA [9]. Similarly, it was recommended for patients with axial diseases not to receive long-term treatment with systematic glucocorticoids and that only glucocorticoid injection direct to the local site of musculoskeletal inflammation may be considered.

Diprospan® is approved dual-acting formulation of suspension of betamethasone phosphate and betamethasone dipropionate for intramuscular of intra-articular use. The phosphate ester of betamethasone is rapidly hydrolyzed and provides immediate short action whereas dipropionate ester is slowly hydrolyzed

* Corresponding author.
E-mail address: a.t.kopylov@gmail.com (A.T. Kopylov).

https://doi.org/10.1016/j.jpba.2017.11.021
0731-7085/

compound stipulating prolong action due to retaining in tissue after injection. Betamethasone is a long-acting glucocorticoid that suppresses release and activity of endogenous mediators of inflammation. One of the first clinical application of betamethasone (BET) was reported more than 40 years ago by Liggins [2]. An optimal effect of a suspension of equal amounts of betamethasone phosphate and acetate on the maturation of the fetus was demonstrated [2] and the desired effect was achieved even with a single injection per day. In 1984 Petersen et al. reported pharmacokinetics in nine patients treated with BET dual-acting suspension formulation [10]. Other research [11] observed the effect of BET in pregnancies and evaluated the effect of maternal body size on the pharmacokinetic parameters.

Metabolic ways of BET processing were poorly investigated for the past twenty years. Some metabolites of BET were found in human urine and sensitivity was limited to detection capability within several hours [11]. A recent paper on BET metabolism had shown the detection of 25 metabolites and a portion of them was rigorously scrutinized including characterization of detection window [12]. The excretion research was conducted on single healthy volunteer and BET was observed as the main compound in human urine after intramuscular administration of a suspension of betamethasone sodium phosphate and betamethasone acetate. Its detection window extended up to 96 h with maximum excretion rate was reached for the first 24 h [12]. However, excretion profile and pharmacokinetic parameters of BET can be different depending on the route of administration [13]. Nevertheless, despite the fact that decades have passed from the first mentioning about BET, discussion on efficiency and, most importantly, the safety of this drug is still in progress [14]. One of the reasons of urgency is that most investigations of BET influence [2,3,10,12] were conducted on a small group of volunteers, thus, the results of such studies cannot be translated into large groups of patients. On the other hand, the number of studies of BET pharmacokinetics in patients with RA or AS is very limited. The existing BET study on patients with RA declares long-lasting inflammatory control [15] and a minimum cumulative effect [15,16]. Since optimum treatment strategies for RA and AS have not yet been established, initial monotherapy versus combination therapy is still debatable.

The goal of the presented study is monitoring of BET excretion in patients with a verified diagnosis of rheumatoid arthritis (RA) or with ankylosing spondylitis (AS). The assay was conducted during 28 days after intramuscular administration of dual-acting formulation to investigate the rate of BET excretion from the body system. The presence of β-phase of elimination and multiple peaks of maximum concentration were observed in this study for several participants. The phenomenon suggested being relevant with body weight and dependent on the background of continual combinatory therapy by non-steroidal drugs. In addition, the effect of BET was controlled through inspection of selected values of a routine blood test.

2. Materials and methods

2.1. Chemicals and reagents

Betamethasone (trademark Vetranal®) analytical standard was purchased from Sigma (Seeize, Germany). Deuterated betamethasone (d5-BET) was purchased from Toronto Research Chemicals (Toronto, Canada). Enzyme β-glucuronidase (type *E. Coli K12*) was purchased from Roche Diagnostics (Mannheim, Germany). Analytical grade sodium di-hydrogen phosphate monohydrate, disodium hydrogen phosphate (anhydrous), potassium carbonate and ethyl acetate were obtained from AppliChem Panreac (Darmstadt, Germany). Methanol (HPLC and spectrophotometry grade) was purchased from J.T. Baker (the Netherlands). Acetonitrile (HPLC grade) was obtained from Fisher Chemical (Loughborough, the UK). Trifluoroacetic acid Reagent Plus®, 99% was from Sigma (St. Louis, MO, USA), formic acid, 98+% was from Acros Organics (Germany). Glass vials (22 mL nominal volume) with screw caps were purchased from Agilent (Paolo Alto, CA, USA). Water (TOC < 3 ppb, 18.2–18.5 mΩ*cm) was obtained from Milli-Q Integral 3 purification system, Millipore S.A.S (France).

2.2. Ethical considerations

An open-comparative prospective cohort study recruited 20 volunteers for assay group (10 patients with rheumatoid arthritis, 10 patients with ankylosing spondylitis) and 20 healthy volunteers for a control group who had not received systemic or local corticosteroids in the last 12 weeks before the screening visit.

All subjects from the assay group and the control group gave written informed consent to participate in the study before the screening. The study was approved by the Local Ethics Committee of V.A. Nasonova Research Institute of Rheumatology and performed in accordance with the WMA Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subject (revised Fortaleza, 2013) and Good Clinical Practice Guidelines approved by the Russian Ministry of Health.

2.3. Clinical study, sample collection and handling

Subjects from the control group were included in the study if they were judged healthy based on their medical history, physical examination, hepatic, renal, respiratory, cardiac, gastrointestinal, complete blood count, and serum chemistry results. In addition, subjects were required to be medication free, including over-the-counter drugs, for 7 days before beginning the study. Subjects were excluded if they tested positive for HIV or hepatitis B and C viruses. Subjects were also excluded if they were prescribed steroidal drugs for previous 12 months. Control group of volunteers comprised of 20 subjects (10 male and 10 female) with an average age of 28.63 ± 3.27 years old, the body weight of 71.05 ± 13.95 kg and BMI (body-mass index) from 18.34 to 26.58 kg/m^2; subjects of the control group did not receive betamethasone for this study.

Assay group of the clinical study consisted of 20 subjects in the age of 29.61 ± 5.33 years old with average body weight of 68.78 ± 10.59 kg and BMI ranged from 17.40 to 28.40 kg/m^2 (Appendix A). All subjects of the assay group have the confirmed diagnosis of rheumatoid arthritis or ankylosing spondylitis and underwent routine therapeutic treatment including the prescription of non-steroidal anti-inflammatory drugs during observation. The drugs taken by subjects of the assay group are indicated in Appendix A.

Following an overnight fasting, subjects of the assay group were administrated a single dose (1 mL) of the dual-acting formulation of suspension comprised betamethasone dipropionate 5 mg/mL and betamethasone disodium phosphate 2 mg/mL (totally 3.7 mg of BET equivalence) (trademark Diprospan®, Schering-Plough Labo N.V. Belgium). The drug was delivered by intramuscular using sterile syringe into upper outer quadrant of buttocks. Subjects from the control group were not administrated by the drug and used as a negative control group.

Urine samples of the assay group were collected before administration the drug (pre-dose) and after 4 days (96 h), 7 days (168 h), 14 days (336 h), 21 days (504 h) and up to 28 days (672 h) after administration. Initially, urine samples were collected in a volume of 100–120 mL in sterile plastic cans in the morning between 8 and 10 a.m., and split in three aliquots of 20–30 mL each. Aliquots were stored at −20 °C upon sample preparation and analysis were performed. The total time of handling per a sample ranged from 5 to