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Perspective

Selective glucocorticoid receptor modulation inhibits cytokine responses in a canine model of mild endotoxemia

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ABSTRACT

Selective glucocorticoid receptor modulators (GRMs) promise to reduce adverse events of glucocorticoids while maintaining anti-inflammatory potency. The present study tested the anti-inflammatory activity of two novel non-steroidal GRMs (GRM1: BI 607812 BS, GRM2: BI 653048 BS*H3PO4) in comparison to prednisolone in a canine model of low dose endotoxemia. This study compared the anti-inflammatory and pharmacokinetic profile of escalating daily oral doses of GRM1 (1, 2.5, 5 and 10 mg/kg) and GRM2 (0.1, 0.25 and 1 mg/kg) with prednisolone (0.25 and 0.5 mg/kg) and placebo after intravenous infusion of endotoxin (0.1 μg/kg) to Beagle dogs. This was followed by a 14-day evaluation study of safety and pharmacokinetics. Endotoxin challenge increased TNF-α ~2000-fold and interleukin-6 (IL-6) 100-fold. Prednisolone and both GRMs suppressed peak TNF-α and IL-6 by 71–82% as compared with placebo. The highest doses of GRM1 and GRM2 reduced the mean body temperature increase by ~30%. The endotoxin-induced rise in plasma cortisol was strongly suppressed in all treatment groups. Pharmacokinetics of both GRMs were non-linear. Adverse effects of endotoxemia such as vomiting were mitigated by GRM2 and prednisolone, indicating an antiemetic effect. During the 14-day treatment period, the adverse event profile of both GRMs appeared to be similar to prednisolone. Both GRMs had anti-inflammatory effects comparable to prednisolone and showed good safety profiles. Compounds targeting the glucocorticoid receptor selectively may provide an alternative to traditional glucocorticoids in the treatment of inflammatory disease.

1. Introduction

Glucocorticoids are potent anti-inflammatory drugs, commonly used in many different acute and chronic diseases. On the downside, several adverse effects limit their therapeutic potential and nearly every organ system can be affected. Undesirable effects on vasculature [1], adipose tissue [2], glucose- [3] or bone metabolism [4] cause considerable morbidity and mortality. Glucocorticoids mediate their anti-inflammatory action predominantly through the glucocorticoid receptor (GR). The GR is a ligand-dependent transcription factor residing in the cytosol [5]. Upon activation, the GR has activating and inhibitory effects on gene-transcription [6]. The anti-inflammatory action is mediated through the inhibition of pro-inflammatory transcription factors whereas most of the unfavourable effects are associated with induction of gene-transcription. Advances in the understanding of molecular mechanisms have led to the discovery of compounds that target selective gene expression [7]. Ideally, such compounds have similar anti-inflammatory activity as conventional glucocorticoids, but should come with a reduced side effect profile [8]. In recent years, several promising molecules have been developed [9–12]. After passing through in vitro pharmacologic studies, a new anti-inflammatory compound is tested in animal models for in vivo activity [13]. We have recently demonstrated that the infusion of low dose endotoxin (lipopolysaccharide; LPS) induces a release

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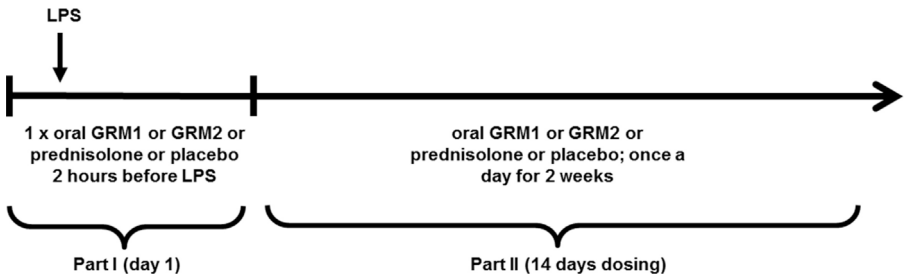

Fig. 1. Schematic of the experimental design. On the first day 80 healthy Beagles received escalating doses of oral GRM1 or GRM2 or prednisolone or placebo (8 per group). This was followed by iv. endotoxin infusion 2 h after drug administration. On the next day a safety and pharmacokinetic evaluation phase (Part II) of multiple doses was initiated and dogs received allocated treatment for 14 days (once daily, without endotoxin).

of the pro-inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in Beagle dogs [14] as seen in human endotoxemia [15]. Moreover, investigation of the standard glucocorticoid prednisolone showed similar pharmacokinetics and pharmacodynamics compared to human studies [14]. Thus, infusion of low dose endotoxin (0.1 μg LPS/kg) provides a tool for testing new anti-inflammatory compounds in a canine model of systemic inflammation. In a recently published series two novel selective glucocorticoid receptor modulators (GRM1: BI 607812 BS and GRM2: BI 653048 BS*H3PO4) were identified who maintained anti-inflammatory activity in vivo in a collagen induced arthritis mouse model with the potential for reduced side effects compared to the widely used synthetic glucocorticoid prednisolone [10–12]. The present study compared the anti-inflammatory activity of these selective glucocorticoid receptor modulators with prednisolone and placebo in a canine model of endotoxemia. This was followed by a 14-day evaluation study of safety and pharmacokinetics.

2. Materials and methods

The study was performed based on Good Laboratory Practice Regulations of the European Commission and the OECD Principles of Good Laboratory Practice and consisted of two parts: Part I was a single dose lipopolysaccharide (LPS) cytokine evaluation study, followed by, part II, a 14-day evaluation of the safety and pharmacokinetics of GMR1, GMR2, prednisolone and placebo (Fig. 1).

The study was conducted in eighty male Beagle dogs (age ranging from 10 to 29 months, weighing 6.9–13.0 kg; from an identical genetic pool). The study was approved by the competent ethics committee, and reported in accordance with the ARRIVE guidelines [16,17]. Dogs were allocated to 10 different study groups (n = 8 per group) employing a pseudo-random body weight stratification procedure that yielded groups with approximately equal mean body weight. On study day 1 all animals received 0.1 μg kg^{-1} body weight (bw) lipopolysaccharide (LPS, Escherichia coli 0111.B4; Sigma-Aldrich Chemie GmbH; Taufkirchen, FRG) diluted in 0.9% NaCl solution 0.1 mL kg^{-1} and injected intravenously as a bolus. This dose was chosen based on previous experiments [14]. LPS was a lyophilised powder and the test item solution was freshly prepared on the administration day adjusted to each animal's current body weight in a total volume of 0.1 mL kg^{-1} bw 0.9% NaCl (B. Braun Melsungen AG, Melsungen, FRG). On days 1–15 the animals of the groups 2–5 received 1, 2.5, 5 and 10 mg/kg GRM1, respectively, the animals of groups 6–8 received 0.1, 0.25 and 1 mg/kg GRM2, respectively and the dogs of groups 9 and 10 received 0.25 and 0.5 mg/kg prednisolone, respectively. Chemical structure of GRM1 (MW 395 g/mole) and GRM2 (MW 515 g/mole) have been published (GRM1: compound 21 [11] and GRM2: compound (R)-39 [12]) and were provided by Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, USA. The doses of the test items given were based on biologic data (human in vitro, animal PK and in vivo pharmacology) [11,12]. On day 1, test and reference drugs were administered orally 2 h prior to intravenous LPS infusion. The test item formulations were freshly prepared once or twice per week adjusted to each animal's current body weight once weekly in vehicle [PEG400/water (75/25% m/m)]. An equal volume of vehicle was given as placebo in group 1. Drinking water was offered ad libidum and food (40 g/kg bw ssniff Hd-H V3234, Spezialdiäten GmbH, Soest, Germany) was served once daily for two hours (up to 8 h in case of poor appetite). The dogs were kept singly or by twos in kennels (including an inside yard and outside yard with a total floor space of total 9 m^2) maintained at a temperature of 22 °C ± 3 °C (maximum range). The adverse event profile was assessed by body weight, thorax circumference, urine production, laboratory tests (hematology, blood chemistry, and coagulation), appearance of clinical sings of systemic toxicity and cortisol suppression, C-Peptide serum levels, osteocalcin levels, served as surrogate safety parameters. At the end of the study, no further examinations were performed and the animals were returned to the stock as a source of plasma or serum samples, e.g. for method development purposes only.

2.1. Blood sampling and laboratory measurements

Blood samples were taken in order to obtain venous blood from the saphenous or cephalic vein of the hind or forelimb for analysis. Concentrations of TNF-α, IL-6, C-reactive protein (CRP), cortisol, and insulin, C-peptide, osteocalcin were measured by using specific enzyme-immunoassays following the instructions of the manufacturer (TNF-α, Quantikine® Canine TNF-α [R&D Systems, Minneapolis, USA]; IL-6, Quantikine® Canine IL-6 [R&D Systems, Minneapolis, USA]; CRP, Canine C-Reactive Protein ELISA Kit [BD Bioscience, San Diego, USA]; osteocalcin, CANINE Osteocalcin ELISA Kit [Cusabio Biotech, Wuhan, Hubei, China]). Plasma leves of GRM1, GRM2, prednisolone and cortisol were measured with liquid chromatography-tandem mass spectrometry by the Department of Bioanalytics of the Nuvisan GmbH (Neu-Ulm, Germany). Sampling times for cytokine determination were on day 1 (-2 [pre-dose], 0 [LPS], 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 22 h relative to LPS infusion) and for biomarker determination on day 2 (0 [pre-dose] and 2 h), day 9 (0 h [pre-dose]), day 15 (0 h [pre-dose] and 2 h relative to dosing). Sampling times for pharmacokinetics, cortisol and prednisolone: on day 1 (0 [pre-dose], 0.25, 0.5, 1, 1.5, 2 [LPS], 4, 6, 8 and 12 h relative to dosing), and after the 14-day treatment period (part II, day 15 [0,0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 36 and 48 h] relative to dosing).

2.2. Pharmacokinetics (PK)

Pharmacokinetic data of GRM1, GRM2, and prednisolone were determined from plasma concentrations and included maximum concentration (C_{max}), half-life ($t_{½}$), time of the last quantifiable analyte concentration t_{last} (h), time to reach maximum concentration