European Journal of Pharmacology 803 (2017) 179–186

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Full length article

JTP-117968, a novel selective glucocorticoid receptor modulator, exhibits improved transrepression/transactivation dissociation

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A R T I C L E I N F O

Keywords:
Selective glucocorticoid receptor modulator (SGRM)
Transrepression
Transactivation
Non-steroidal
JTP-117968

A B S T R A C T

Classic glucocorticoids that have outstanding anti-inflammatory effects are still widely prescribed for the treatment of various inflammatory and autoimmune diseases. Conversely, glucocorticoids cause numerous unwanted side effects, particularly systemically dosed glucocorticoids. Therefore, selective glucocorticoid receptor modulator (SGRM), which maintains beneficial anti-inflammatory effects while reducing the occurrence of side effects, is one of the most anticipated drugs. However, there have been no SGRMs marketed to date.

The assumption is that there are two major mechanisms of action of glucocorticoids via glucocorticoid receptors, transrepression (TR) and transactivation (TA). In general, the anti-inflammatory effects of glucocorticoids are mostly mediated through TR, while the side effects associated with glucocorticoids are largely caused by TA.

We started to evaluate novel orally available SGRMs that maintain anti-inflammatory effects while minimizing adverse effects by favoring TR over TA. Based on this evaluation, we discovered JTP-117968, (4b'*S*,7′*R*,8a'*S*)-4b'-benzyl-7′-hydroxy-*N*-(2-methylpyridin-3-yl)-7′-(trifluoromethyl)-4b',6′,7′,8′,8a',10'-hexahydro-5′*H*-spiro[cyclopropane-1,9′-phenanthrene]-2′-carboxamide, a non-steroidal SGRM. JTP-117968 has partial TR activity, but exhibits extremely low TA activity. The maximum TR efficacy of JTP-117968 was comparable to its structural analogue, PF-802, (4b*S*,7 *R*,8a*R*)-4b-Benzyl-7-hydroxy-*N*-(2-methylpyridin-3-yl)-7-(trifluoromethyl)-4b,5,6,7,8,8a,9,10-octahydrophenanthrene-2-carboxamide, which is the active form of Fosdagrocorat that has been developed clinically as a first-in-class orally available SGRM. Remarkably, the TA activity of JTP-117968 was much weaker than PF-802 not only in *in vitro* assays, but also in *in vivo* mice experiments.

These findings indicate that JTP-117968 exhibits improved TR/TA dissociation because the compound has significantly lower TA activity compared with an already reported SGRM. Therefore, JTP-117968 is expected to be a useful compound for evaluating ideal SGRMs in the future.

1. Introduction

Glucocorticoids have been widely prescribed for diverse inflammatory and autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, autoimmune hepatitis and nephrotic syndrome, since Lewis Hasting Sarett succeeded in synthesizing cortisone at Merck Research Laboratories (Sarett, 1946). Conversely, glucocorticoids cause numerous undesired side effects, including diabetes, osteoporosis, central obesity and hypertension, particularly during systemic administration (Schäcke et al., 2002; van der Goes et al., 2010). By developing topical application glucocorticoids (inhaled formulations, ointments, and eye drops, etc.), these side effects have been reduced significantly. However, the systemic administration of glucocorticoids is still needed to regulate most autoimmune diseases. The side effects mentioned above limit the dose and duration of treatment with glucocorticoids. Therefore, SGRMs, which maintain beneficial anti-inflammatory effects but reduce the occurrence of side effects, are one of the most highly anticipated drugs in the clinical field.

Glucocorticoids regulate gene expression via glucocorticoid receptors. The assumption is that there are two major mechanisms of action of glucocorticoid receptors. One major mechanism is via TA, through the activation of mRNA expression of various molecules such as

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http://dx.doi.org/10.1016/j.ejphar.2017.03.057
Received 21 October 2016; Received in revised form 14 March 2017; Accepted 29 March 2017
Available online 31 March 2017
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tyrosine aminotransferase (TAT) (Jantzen et al., 1987) via glucocorticoid response elements (GRE). Another mechanism is via TR, through the inhibition of binding of transcription factors, such as Nuclear Factor kappa B and Activator Protein −1 (Barnes, 1998, 2006), to the transcription site of mRNA that is independent of GRE. In general, the anti-inflammatory effects of glucocorticoids are mostly mediated through TR, while the side effects of glucocorticoids are largely caused by TA.

Over the last couple of decades, enormous efforts have been made in the development of SGRMs that maintain anti-inflammatory effects while minimizing adverse effects by favoring TR over TA activity via glucocorticoid receptors (Lopez et al., 2008; Schäcke et al., 2004; van Lierop et al., 2012). Several SGRM drugs are being developed clinically (Baiula and Spampinato, 2014; Hu et al., 2011; Schäcke et al., 2009; Sundahl et al., 2015; Zhang et al., 2009); however, no SGRMs have been approved and marketed to date.

We started to evaluate novel SGRMs that retain anti-inflammatory effects while minimizing adverse effects by favoring TR over TA. Based on this evaluation, we discovered JTP-117968, (4b'*S*,7′*R*,8a'*S*)-4b'-benzyl-7′-hydroxy-*N*-(2-methylpyridin-3-yl)-7′-(trifluoromethyl)-4b',6′,7′,8′,8a',10'-hexahydro-5′*H*-spiro[cyclopropane-1,9′-phenanthrene]-2′-carboxamide, a non-steroidal SGRM. JTP-117968 was found to be a more potent and selective glucocorticoid receptors ligand than other steroidal receptors. This compound maintained partial TR activity *in vitro*, while demonstrating extremely low TA activity.

JTP-117968 has a similar structure to PF-802, (4b*S*,7 *R*,8a*R*)-4b-Benzyl-7-hydroxy-*N*-(2-methylpyridin-3-yl)-7-(trifluoromethyl)−4-b,5,6,7,8,8a,9,10-octahydrophenanthrene-2-carboxamide, an active form of Fosdagrocorat that has been developed on the Phase 2 clinical stage as a first-in-class orally available SGRM (Buttgereit et al., 2014). The maximum TR efficacy of JTP-117968 was comparable to PF-802. Notably, the TA activity of JTP-117968 was much lower than PF-802 not only in *in vitro* assays, but also in *in vivo* experiments.

These findings indicate that JTP-117968 exhibits improved TR/TA dissociation because the compound has significantly lower TA activity compared with an already reported SGRM. Hence, JTP-117968 is expected to be a useful compound for evaluating ideal SGRMs in the future.

2. Materials and methods

2.1. Chemicals and reagents

JTP-117968 and PF-802 (Fig. 1) were synthesized at the Central Pharmaceutical Research Institute within Japan Tobacco Inc. (Osaka, Japan). Test compounds were dissolved in dimethyl sulfoxide (DMSO) *in vitro*. All other chemicals were standard reagent grade.

2.2. Animal housing and care

Female BALB/c mice were purchased from Japan SLC (Shizuoka, Japan). Mice were maintained with free access to water and normal chow diet (CRF-1, Charles River Japan). Animals were housed under specific pathogen-free conditions in a room controlled for temperature at 23 ± 3 °C and humidity at 55 ± 15% in 12-h light/dark cycles (lights on from 8:00 a.m. to 8:00 p.m.). All of the procedures were performed according to guidelines from Japan Tobacco's Animal Care Committee.

2.3. Nuclear receptor (NR) competitor assay

The competitive binding assays for glucocorticoid receptors, progesterone receptors, androgen receptors, estrogen receptor alpha and beta were performed using PolarScreen ™ competitor assay kits, which were utilized in accordance with the manufacturer's instructions (Thermo Fisher Scientific Inc., Carlsbad, CA). Briefly, each NR was added to each fluorescent ligand, Fluormone ™ tracer, in the presence of test compounds in 96-well black round bottom plates. The plates were mixed and incubated for at least 2 h. The fluorescence polarization value of each well was then subsequently measured using Multilabel Reader Envision ™ (PerkinElmer, Waltham, MA) at 531 nm excitation and 595 nm emission. Test compounds that displaced the Fluormone ™ tracer from the NR/Fluormone ™ tracer complex caused decreases in polarization. Displaced Fluormone ™ tracer tumbled rapidly, resulting in low polarization values. The decrease in polarization value was used to determine the relative affinity of the test compounds for each NR.

2.4. Mineralocorticoid receptor reporter gene assay

The agonist/antagonist activities of test compounds against mineralocorticoid receptors were evaluated using the Human MR Reporter Assay System (Indigo Biosciences, State College, PA), which was utilized in accordance with the manufacturer's instructions with slight modifications. Briefly, in the agonist assay, mineralocorticoid receptor reporter cells that incorporated the cDNA encoding luciferase were dispensed into wells of 96-well assay plates, after which test compounds were added immediately. Following a 22–24 h incubation in a 37 °C, humidified 5% CO_2 incubator, the Steady-Glo® Reagent prepared from the Steady-Glo® Luciferase Assay System (Promega, Madison, WI) was dispensed. Luciferase activity was measured using Multilabel Reader Envision ™. Aldosterone was used as the reference agonist for the mineralocorticoid receptors agonist assay. To evaluate for antagonist activity, mineralocorticoid receptor reporter cells were incubated with or without test compounds for approximately 0.5 h. Aldosterone was then subsequently dispensed into each well (final concentration; 100 pM) and incubated for 22–24 h, after which Luciferase activity was measured using the same method as the agonist assay. Spironolactone was used as the reference antagonist for the mineralocorticoid receptor antagonist assay.

2.5. Interleukin-6 (IL-6) release from A549 cells

Human A549 lung epithelial cells (American Type Culture Collection, Manassas, VA) were cultured in Ham's F12K (Wako Pure Chemical Industries, Ltd., Osaka, Japan) with 100 U/mL penicillin-streptomycin (Thermo Fisher Scientific Inc., Carlsbad, CA) and 10%

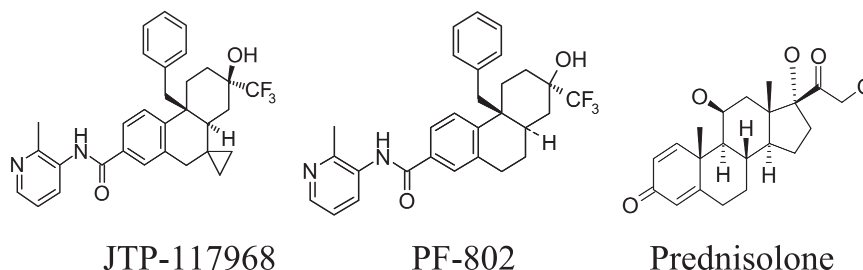

Fig. 1. Chemical structures of JTP-117968, PF-802 and prednisolone.

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