Title page

JTP-117968, a novel selective glucocorticoid receptor modulator, exhibits significant anti-inflammatory effect while maintaining bone mineral density in mice

Takafumi Kurimoto[a,b*], Isao Tamai[a], Takashi Nakagawa[a], Atsuko Miyai[a], Yasuo Yamamoto[a], Yoshinori Kosugi[a], Katsuya Deai[a], Takahiro Hata[a], Takeshi Ohta[c], Mutsuyoshi Matsushita[a], Takahisa Yamada[b]

[a]Central Pharmaceutical Research Institute, Japan Tobacco Inc., Osaka, Japan

[b]Faculty of Agriculture, Department of Agrobiology, Niigata University, Niigata, Japan.

[c]Laboratory of Animal Physiology and Functional Anatomy, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

*Corresponding author

Takafumi Kurimoto

Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-1 Murasaki-cho, Takatsuki, Osaka 569-1125, Japan

Tel.: +81 72 681 9700

Fax: +81 72 681 9783

E-mail: takafumi.kurimoto@jt.com

Abstract

Classic glucocorticoids have been prescribed for various inflammatory diseases, such as rheumatoid arthritis, due to their outstanding anti-inflammatory effects. However, glucocorticoids cause numerous unwanted side effects, including osteoporosis and diabetes. Hence, selective glucocorticoid receptor modulators (SGRMs), which retain anti-inflammatory effects with minimized side effects, are among the most anticipated drugs in the clinical field.

The assumption is that there are two major mechanisms of action via glucocorticoid receptors, transrepression (TR) and transactivation (TA). In general, anti-inflammatory effects of glucocorticoids are largely due to TR, while the side effects associated with glucocorticoids are mostly mediated through TA. We previously reported that JTP-117968, a novel SGRM**,** maintained partial TR activity while remarkably reducing the TA activity. In this study, we investigated the anti-inflammatory effect of JTP-117968 on a lipopolysaccharide (LPS) challenge model and collagen-induced arthritis (CIA) model in mice. Meanwhile, we tested the effect of JTP-117968 on the bone mineral density (BMD) in mouse femur to evaluate the side effect. Based on the evaluation, JTP-117968 reduced the plasma levels of tumor necrosis factor α induced by LPS challenge in mice significantly. Remarkably, CIA development was suppressed by JTP-117968 comparably with prednisolone and PF-802, an active form of fosdagrocorat that has been developed clinically as an orally available SGRM. Strikingly, the side effect of JTP-117968 on mouse femoral BMD was much lower than those of PF-802 and

prednisolone. Therefore, JTP-117968 has attractive potential as a new therapeutic option against inflammatory diseases with minimized side effects compared to classic glucocorticoids.

Keywords

Selective glucocorticoid receptor modulator; JTP-117968; Collagen-induced arthritis model;

Glucocorticoid-induced osteoporosis; PF-802; Prednisolone

1. Introduction

Classic glucocorticoids, such as prednisolone and dexamethasone, are widely prescribed to treat diverse autoimmune and inflammatory diseases, including rheumatic arthritis and systemic lupus erythematosus. Conversely, classic glucocorticoids cause harmful side effects such as osteoporosis, insulin resistance, and many others, particularly during systemic administration (Schacke et al., 2002; van der Goes et al., 2010). The side effects mentioned above limit the dose and duration of treatment with classic glucocorticoids. Therefore, selective glucocorticoid receptor modulators (SGRMs), which retain beneficial anti-inflammatory effects but reduce the occurrence of side effects, are among the most anticipated drugs in the clinical field.

Glucocorticoids regulate a wide variety of gene expressions through glucocorticoid receptor which controls gene transcriptions via multiple and complex mechanisms (Barnes, 2006; Hudson et al., 2013). The assumption is that there are two major mechanisms of action of glucocorticoid receptor: one being via transactivation (TA), through activation of mRNA expression of various molecules such as tyrosine aminotransferase (Jantzen et al., 1987) via glucocorticoid response elements (GRE), and the other via transrepression (TR), through interference in the binding of transcription factors, such as Nuclear Factor kappa B (NFκB) (Barnes, 1998, 2006), to a transcription site of mRNA that is independent of GRE. Although several reports suggest that some anti-inflammatory proteins are induced by TA activity (Barnes, 2006; Moreno, 1997; Reuter et al., 2012; Shipp et al., 2010), it is

generally believed that anti-inflammatory effects of classic glucocorticoids are largely due to TR, while the problematic side effects associated with classic glucocorticoids are mediated through TA. Over the past few decades, numerous efforts have been made in the development of SGRMs that retain anti-inflammatory effects while minimizing side effects by favoring TR over TA activity via glucocorticoid receptor (Lopez et al., 2008; Schacke et al., 2004; van Lierop et al., 2012). One of the drug candidates, fosdagrocorat, has been developed clinically as an orally available SGRM that was reported to show efficacy in patients with moderate to severe rheumatoid arthritis comparable to prednisone (Buttgereit et al., 2019). However, none of these SGRMs have been approved and marketed to date.

Recently, we reported that 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 that exhibited improved TR/TA dissociation (Kurimoto et al., 2017). JTP-117968 maintained partial TR activity while remarkably reducing the TA activity. The maximum TR efficacy of JTP-117968 was slightly lower than 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. Notably, the TA activity of JTP-117968 was much weaker than those of prednisolone and PF-802.

In this study, we evaluated the anti-inflammatory effect of JTP-117968 on a lipopolysaccharide (LPS) challenge model and collagen-induced arthritis (CIA) model in mice. Meanwhile, we tested the effect of this compound on the bone mineral density (BMD) in mouse femur to evaluate the side effect. Furthermore, we evaluated the effect of JTP-117968 on mRNA expression of Dickkopf-1 (Dkk-1), which negatively regulates bone formation, in human primary osteoblasts. Prednisolone and PF-802 were also examined, as reference compounds in this study.

2. Materials and methods

2.1. Chemicals and reagents

JTP-117968 and PF-802 (Fig. 1) were synthesized in the Central Pharmaceutical Research Institute within Japan Tobacco Inc. (Osaka, Japan). The purities of both synthesized compounds were greater than 95% (by nuclear magnetic resonance analysis). Prednisolone was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). PF-802 and prednisolone were used as reference compounds.

Dimethyl Sulfoxide (DMSO) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan) and used as a vehicle in *in vitro* experiments. Metolose® SM-1500 (methylcellulose) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan) and its 0.5% aqueous solution was used as a vehicle in *in vivo* experiments. All other chemicals were standard reagent grade.

2.2. Animal housing and care

Female BALB/c mice and male DBA/1JNCrlj mice were purchased from Japan SLC (Shizuoka, Japan) and Charles River Laboratories (Yokohama, Japan), respectively. Mice were maintained with free access to water and normal chow diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan). Animals were housed under specific pathogen-free conditions in a room with controlled temperature of 23 ± 3°C and humidity of 55 ± 15% in 12-h light/dark cycles (lights on from 8:00 AM to 8:00 PM). The animal study protocol was approved by the Institutional Animal Care and Use Committee of the

Central Pharmaceutical Research Institute, Japan Tobacco Inc. (Approval number 11120502, 11120601 and 12030602).

2.3. Mouse LPS challenge model

Female BALB/c mice (about 20 g at dosing) were dosed orally with vehicle (0.5% Methylcellulose) or test compound 1 hour before a challenge with an intravenous dose of 0.25 mg/kg of *Escherichia coli* LPS (from strain O111:B4, Sigma-Aldrich Co.) dissolved in phosphate-buffered saline. Blood samples were collected from mice 90 min after LPS challenge and the plasma fractions were stored at −80°C until assays were performed. Plasma levels of tumor necrosis factor α (TNFα) were determined by Mouse TNFα Immunoassay (R&D systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions. No TNFα was detected in the plasma from mice without LPS challenge.

2.4. Mouse collagen-induced arthritis model

The experiment was performed essentially as described previously (Yamaguchi et al., 2012). Briefly, bovine type II collagen (CII, Collagen Research Center, Tokyo, Japan) was dissolved in 0.01 M acetic acid at a concentration of 2 mg/ml and then emulsified in an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA). Seven-week-old male DBA/1JNCrlj

mice were immunized by intradermal injection at the base of the tail with 100 μg CII emulsion. The day of the first immunization was designated day 1. After 21 days, the mice were randomized into matched groups (9 or 10 mice per group) based on body weight, and the mice received the same amount of the CII emulsion to induce arthritis (day 22). Vehicle (0.5% Methylcellulose) or test compound was given orally once daily from day 22 to day 35. The arthritic score for the mice was obtained by summing the visual severity grade of each limb, in which swelling of digits and entire paw was scored as follows (maximum score for each limb was 4): for the swelling of digits (0, no swelling; 1, one swollen digit; 2, two or more swollen digits), for the swelling of entire paw (0, no swelling; 1, mild swelling; 2, severe swelling of the entire paw). The score was obtained in a blind manner. Arthritic scores of individual mice were represented as an average score of 4 limbs. On the day following the last administration, the mice were euthanized. The spleen of each mouse was collected after connective tissues and debris were removed. The relative weight of spleens was calculated based on absolute weight of spleens and body weight at sacrifice.

2.5. Measurement of BMD in normal BALB/c mice after 28 days of repeated oral dosing

The experiment was performed essentially as described previously (Marenzana et al., 2011). Female BALB/c mice were 12 weeks of age at the start of the studies. Vehicle (0.5% Methylcellulose) or test compound was given orally once daily for 28 days. On the day following the

last administration, the mice were euthanized. Left leg of each mouse was collected after connective tissues, muscles, and debris were removed to determine the BMD of femur bones. The left femur was excised and cleaned of excess soft tissue. The BMD of each femur was measured by peripheral quantitative computed tomography using a LaTheta LCT-100A micro-CT scanner (Aloka, Tokyo, Japan) with a slice thickness of 1 mm.

2.6. Induction of Dkk-1 mRNA in human primary osteoblasts

Cryopreserved human primary osteoblasts were purchased from PromoCell (Heidelberg, Germany). Cells were thawed and cultured in PromoCell Osteoblast Growth Medium (C-27001). A total of 3 x 10^4 cells in the medium were seeded onto 24-well plates. After 8 hours of incubation in a 37°C, humidified 5% CO_2 incubator, medium for cells was switched to PromoCell Osteoblast Basal Medium (C-27010) and the cells were incubated with or without compounds (0.1% DMSO) for 24 hours. Total RNA was then extracted from cell lysates using GenElute ™ Mammalian Total RNA Miniprep Kit (Sigma-Aldrich Co.). Quantitative real-time polymerase chain reaction was performed using Taqman® RNA-to-Ct ™ 1-Step kits on StepOnePlus ™ (Thermo Fisher Scientific Inc., Waltham, MA, USA), which were used in accordance with the manufacturer's instructions. Dkk-1 (Hs00183740_m1) and GAPDH (Hs99999905_m1) primer/probe sets were obtained from Thermo Fisher Scientific Inc. The induction of human Dkk-1 mRNA by vehicle (0.1% DMSO) was defined

as the control at 100%. The percent activation for each compound concentration was calculated relative to the control.

2.7. Statistical analysis

Data are expressed as the mean ± standard deviation for the indicated number of samples. The statistical analysis was performed with statistical software, StatLight 2000 (Yukms Corp., Tokyo, Japan). Student's *t*-test was performed and homogeneity was confirmed by F-test. In instances in which homogeneity was not confirmed by F-test, Welch's *t*-test was to be performed. Dunnett's multiple comparison test was performed in the multiple-group study provided that homogeneity was confirmed by Bartlett's homoscedasticity test. Steel's multiple comparison test was used in the multiple-group study for heteroscedastic data confirmed using Bartlett's homoscedasticity test.

3. Results

3.1. Effect of JTP-117968 in LPS challenge model mice

JTP-117968 is a non-steroidal and novel glucocorticoid receptor selective ligand that exhibits partial TR activity and extremely low TA activity (Kurimoto et al., 2017). It is well known that LPS-induced TNFα release in mouse plasma is inhibited by dosing classic glucocorticoids such as prednisolone (Mihara et al., 2008), and NFκB, one of the major targets of TR, mainly regulates the TNFα release induced by LPS challenge (Higuchi et al., 2006). Therefore, we examined the effect of JTP-117968 against TNFα release elicited in LPS-injected mice to evaluate *in vivo* TR activity. In our experiment, we confirmed the inhibitory effect of prednisolone on TNFα release as reported previously (Mihara et al., 2008) (Supplementary Fig. 1). The TNFα level in plasma 1.5 hours after injection of LPS to BALB/c mice was significantly inhibited with JTP-117968 (49% and 51% inhibition against vehicle control in 30 mg/kg and 100 mg/kg JTP-117968 groups, respectively; Fig. 2). It is reported that PF-802, the structural analogue of JTP-117968, almost completely inhibits the LPS-induced TNFα release in adrenalectomized mouse plasma (Hu et al., 2011). In our study, 30 mg/kg of PF-802 inhibited the LPS-induced TNFα release significantly (approximately 70% inhibition against vehicle control; Fig. 2). We additionally examined the dose-response curve of PF-802 in mouse LPS challenge model. In contrast to the previous report (Hu et al., 2011), the maximum *in vivo* TR activity of PF-802 was obviously lower than the activity of prednisolone

(Supplementary Fig. 2).

3.2. Effect of JTP-117968 in mouse CIA model

Next, we investigated the effect of JTP-117968 on a chronic autoimmune disease model in which classic glucocorticoids exhibit the efficacy. The mouse CIA model is well accepted as an animal model of human rheumatoid arthritis, and it is reported that the development of arthritis in the model is suppressed by dosing classic glucocorticoids (Mihara et al., 2008; van Lierop et al., 2012). Hence, we tested JTP-117968 in this disease model. CII, emulsified with Freund's complete adjuvant, was injected intradermally at the base of the tail of DBA/1JNCrlj mice on day 1 and day 22. The paw swelling was sequentially scored from day 22 to day 36. The compounds were orally administered once a day from day 22 to day 35. First, we confirmed that the dose-related inhibitory effect of prednisolone on the development of arthritis was similar to that reported as mentioned above (-7%, 70%, and 93% inhibition on day 36 against vehicle control in 0.03 mg/kg, 0.3 mg/kg, and 3 mg/kg prednisolone groups, respectively, with a 50% effective dose (ED_{50}) value of 0.16 mg/kg; Supplementary Fig. 3A, Supplementary Fig. 3B). Remarkably, JTP-117968 suppressed the development of arthritis significantly against vehicle control (51% and 80% inhibition on day 36 against vehicle control in 10 mg/kg and 30 mg/kg JTP-117968 groups, respectively, with an ED_{50} value of approximately 10 mg/kg; Fig. 3A, Fig. 3B). In our study, PF-802 also inhibited the

development of arthritis comparably with the effects of prednisolone (42%, 89%, and 97% inhibition on day 36 against vehicle control in 0.03 mg/kg, 0.3 mg/kg, and 3 mg/kg PF-802 groups, respectively, with an ED_{50} value of 0.04 mg/kg; Supplementary Fig. 3A and Supplementary Fig. 3B).

Additionally, we evaluated the relative spleen weight in mouse CIA model, as it is reported that an increase in the relative spleen weight is observed during the development of arthritis (Kwon et al., 2014; Madan et al., 2012; Sohn et al., 2013). In our experiment, we confirmed that the relative spleen weight in the vehicle control group on day 36 was significantly increased compared to the normal group (Fig. 4). JTP-117968 inhibited the increase in the relative spleen weight significantly (78% and 107% inhibition against vehicle control in 10 mg/kg, and 30 mg/kg JTP-117968 groups, respectively, Fig. 4). Also, prednisolone and PF-802 exhibited dose-related inhibitory effect against the increase in the relative spleen weight (4%, 68%, and 104% in 0.03 mg/kg, 0.3 mg/kg, and 3 mg/kg prednisolone groups, respectively, with an ED_{50} value of 0.16 mg/kg; 34%, 96%, and 132% in 0.03 mg/kg, 0.3 mg/kg, and 3 mg/kg PF-802 groups, respectively with an ED_{50} value of 0.05 mg/kg; Supplementary Fig. 4).

3.3. Effect of JTP-117968 on BMD in normal BALB/c mice after 28 days of repeated oral dosing

Classic glucocorticoids are widely prescribed to treat diverse autoimmune and inflammatory diseases due to their phenomenal anti-inflammatory effect. However, long-term systemic dosing of

classic glucocorticoids causes harmful side effects such as osteoporosis. It is reported that daily oral glucocorticoids treatment leads to a reduction in BMD and a rapid increase in the risk of fracture during the treatment period (van Staa et al., 2002). Meanwhile, JTP-117968 is expected to have less side effects, as the TA activity of this compound was extremely lower than that of prednisolone, not only in *in vitro* but also *in vivo* experiments (Kurimoto et al., 2017). Therefore, we next assessed the effect of JTP-117968 on the BMD in normal mouse femur. It is well known that a reduction of the BMD is observed during repeated dosing of classic glucocorticoids to mice (Marenzana et al., 2011; Thiele et al., 2012), similar to glucocorticoid-induced osteoporosis in the clinical field. In our study, we confirmed that prednisolone reduced the BMD in mouse femur after 28 days of repeated oral administration (1.6%, 3.3%, and 7.0% reduction against vehicle control in 0.3 mg/kg, 3 mg/kg, and 30 mg/kg prednisolone groups, respectively; Fig. 5). Strikingly, the reduction of the BMD was not observed in the mouse femur after repeated dosing of JTP-117968 (-0.8% and -1.4% reduction against vehicle control in 3 mg/kg and 30 mg/kg JTP-117968 groups, respectively; Fig. 5). On the other hand, PF-802 showed the tendency to decrease the BMD in mice (-1.2%, 2.1%, and 3.0% reduction against vehicle control in 0.03 mg/kg, 0.3 mg/kg, and 3 mg/kg PF-802 groups, respectively; p=0.09 vs vehicle control in 3 mg/kg PF-802 group; Fig. 5).

3.4. Effect of JTP-117968 on Dkk-1 mRNA expression in human primary osteoblasts

It is reported that glucocorticoid-induced osteoporosis is mainly caused by inhibition of osteoblastic bone formation (Tamura et al., 2004). In human osteoblasts, glucocorticoids enhance the expression of Dkk-1, which negatively regulates bone formation via a TA mechanism (Ohnaka et al., 2004; Wang et al., 2008). Therefore, we evaluated the Dkk-1 induction activity of JTP-117968 in human primary osteoblasts. Based on the evaluation, JTP-117968 barely induced Dkk-1 mRNA expression in comparison with prednisolone (124%, 117%, and 134% induction at 10 nM, 100 nM, and 1000 nM JTP-117968; 206%, 810%, and 1026% induction at 10 nM, 100 nM, and 1000 nM prednisolone against 0.1% DMSO control, respectively; Fig. 6A). Moreover, the Dkk-1 induction activity of JTP-117968 was even lower than that of PF-802 (170% induction at 1000 nM JTP-117968; 190%, 250% and 258% induction at 10 nM, 100 nM, and 1000 nM PF-802 against 0.1% DMSO control; Fig. 6B).

4. Discussion

Classic glucocorticoids, such as cortisol, prednisolone, and dexamethasone have been widely prescribed for various inflammatory and autoimmune diseases due to their phenomenal therapeutic effects since Lewis Hastings Sarett succeeded in synthesizing cortisone at Merck Research Laboratories in 1946 (Sarett, 1946). However, glucocorticoids cause numerous unwanted side effects, including osteoporosis, diabetes, central obesity, muscle proteolysis, hypothalamic-pituitary-adrenal axis suppression, coagulation and psychosis (Johannesdottir et al., 2013; Schacke et al., 2002; Schacke et al., 2004). By developing local application glucocorticoids, such as ointments, eye drops, and inhaled formulations, these side effects were minimized. However, the systemic administration of glucocorticoids is still necessary to regulate the majority of autoimmune diseases, such as rheumatic arthritis, systemic lupus erythematosus, nephrotic syndrome, and autoimmune hepatitis. The use of systemic glucocorticoids is limited by frequent side effects, as previously mentioned. Therefore, SGRMs, which maintain beneficial anti-inflammatory and immunosuppressive effects while reducing side effects, are among the most anticipated drugs in the clinical field.

Over the last few decades, immense efforts have been made to develop SGRMs that favor TR over TA activity via glucocorticoid receptor, such as with the development of ZK-216348 (Schacke et al., 2004), LGD-5552 (Lopez et al., 2008; Miner et al., 2007), Org 214007-0 (van Lierop et al., 2012),

and MK-5932 (Brandish et al., 2014; Bungard et al., 2011). However, these compounds have not reached the clinical stage of development. On the other hand, mapracorat/ZK-245186/BOL-303242-X and AZD7594 have been developed as topical SGRMs in clinical trials (Baiula and Spampinato, 2014; Brown et al., 2019; Schacke et al., 2009; Zhang et al., 2009). Furthermore, as first-in-class orally available SGRMs, fosdagrocorat/PF-04171327 and AZD9567 have been developed in clinical trials (Hegelund Myrbäck et al., 2020; Stock et al., 2009). Repeated dosing of fosdagrocorat was reported to be well tolerated, and an acceptable safety profile was observed in a randomized, placebo-controlled Phase 1 study (Tammara et al., 2013). In addition to the safety profile, fosdagrocorat showed efficacy in rheumatoid arthritis patients with inadequate response to methotrexate (Buttgereit et al., 2019). However, none of these SGRMs have been approved and marketed to date.

Recently, we reported that we discovered JTP-117968, a non-steroidal SGRM that exhibited improved TR/TA dissociation (Kurimoto et al., 2017). JTP-117968 maintained partial TR activity while remarkably reducing the TA activity. The maximum TR efficacy of JTP-117968 was slightly lower than its structural analogue, PF-802, which is the active form of fosdagrocorat. Notably, the TA activity of JTP-117968 was much weaker than those of PF-802 and prednisolone, not only in *in vitro* assays, but also in *in vivo* mice experiments (Kurimoto et al., 2017).

In this study, we examined the anti-inflammatory effect of JTP-117968, which maintained the

partial TR activity. Meanwhile, we tested the effect of JTP-117968, which had extremely reduced TA activity, on the bone metabolism in mice to evaluate the side effect.

First, we confirmed that JTP-117968 showed the significant inhibitory effect on TNFα release elicited in LPS-injected mice, and the effects of 30 mg/kg of JTP-117968 were similar to those of 100 mg/kg of this compound (Fig. 2). It is reported that LPS-induced TNFα release in mice was mainly mediated by NFκB, one of the major targets of TR (Higuchi et al., 2006). We previously reported that the plasma concentration after dosing 100 mg/kg of JTP-117968 was higher than the concentration of 30 mg/kg of this compound (Kurimoto et al., 2017). Therefore, this result indicates that JTP-117968 exhibited partial TR activity in *in vivo* experiments in line with the result from *in vitro* assay we had previously reported (Kurimoto et al., 2017). Moreover, we confirmed that PF-802 inhibited the TNFα release in LPS challenge model, and the maximum efficacy was lower than the effect of 10 mg/kg of prednisolone (Supplementary Fig. 2). It has been reported that the plasma concentration after dosing PF-802 increased in a dose-dependent manner (Kurimoto et al., 2017). Hence, it is assumed that PF-802 also has partial TR activity *in vivo,* in line with *in vitro* experiments as previously reported (Kurimoto et al., 2017).

Second, we evaluated the effect of JTP-117968 on the mouse CIA model, which is well accepted as a chronic autoimmune disease model in which glucocorticoids exhibit the inhibitory effect. Remarkably, JTP-117968 showed an almost completely suppressive effect against the development

of arthritis (Fig. 3A and Fig. 3B). In addition to the arthritis score, JTP-117968 inhibited the increase in the relative spleen weight in mouse CIA model completely (Fig. 4). We also found that PF-802 showed the inhibitory effect on the development of arthritis and the increase in the relative spleen weight in the mouse CIA model comparably with prednisolone (Supplementary Fig. 3A, Supplementary Fig. 3B and Supplementary Fig. 4). Although several reports suggested that some anti-inflammatory proteins, including mitogen-activated kinase phosphatase-1 and Annexin 1, are induced by TA activity via glucocorticoid receptor (Barnes, 2006; Moreno, 1997; Reuter et al., 2012; Shipp et al., 2010), it is evident from our experiment that JTP-117968 with extremely reduced TA activity could exhibit an anti-inflammatory effect comparable to that of classic glucocorticoids. In the clinical trials, fosdagrocorat was demonstrated as showing efficacy in rheumatoid arthritis patients comparable to that of prednisolone (Buttgereit et al., 2019). Hence, JTP-117968, which has partial TR activity, is expected to exhibit anti-inflammatory effect against rheumatoid arthritis comparable to that of classic glucocorticoids. To assess the effectiveness of JTP-117968 against extensive autoimmune disease, further study will be required to evaluate the anti-inflammatory effect of JTP-117968 in other animal disease models, such as spontaneous systemic lupus erythematosus model and anti-glomerular basement membrane antibody-induced glomerulonephritis model.

Next, we examined the effect of JTP-117968, which has exceedingly low TA activity, on the mouse bone metabolism to evaluate the side effect. It is reported that daily oral glucocorticoids treatment

leads to a reduction in BMD and a rapid increase in the risk of fracture during the treatment period (van Staa et al., 2002). Therefore, we evaluated the effect of JTP-117968 on the BMD in normal mouse femur. In our experiment, we confirmed that prednisolone reduced the BMD in mouse femur after 28 days of repeated oral administration as reported previously (Marenzana et al., 2011) (Fig. 5). Strikingly, JTP-117968, which exhibited the almost completely suppressive effect against the development of arthritis in the mouse CIA model, hardly altered the BMD in mouse femur after 28 days of repeated dosing (Fig. 5). Meanwhile, PF-802 showed the tendency to reduce the BMD in mice ($p=0.09$ vs vehicle control in 3 mg/kg PF-802 group, Fig. 5). It is assumed that glucocorticoid-induced osteoporosis is mainly caused by reduction of osteoblastic bone formation (Tamura et al., 2004). In human osteoblasts, classic glucocorticoids increase the expression of Dkk-1, which negatively regulates bone formation via a TA mechanism (Ohnaka et al., 2004; Wang et al., 2008). As JTP-117968 hardly reduced the BMD in mouse femur, we examined the Dkk-1 induction activity of JTP-117968 in human primary osteoblasts. Based on the evaluation, JTP-117968 barely induced Dkk-1 mRNA expression in comparison with prednisolone (Fig. 6A). Remarkably, the Dkk-1 induction activity of JTP-117968 was even lower than that of PF-802 (Fig. 6B). Therefore, JTP-117968, with minimized TA activity, is expected to have less side effects on bone formation in humans compared with classic glucocorticoids and already reported SGRMs.

Moreover, it has been reported that JTP-117968 had neither agonist nor antagonist activity against

mineralocorticoid receptor, unlike classic glucocorticoids (Kurimoto et al., 2017). Hence, a reduction in several side effects associated with glucocorticoids via mineralocorticoid receptor, including hypertension and electrolyte imbalance (Frey et al., 2004), would also be expected during the administration of JTP-117968. Further pharmacological studies will be required to evaluate the effects of JTP-117968 versus classic glucocorticoids in terms of various adverse effects mentioned previously to claim true separation of therapeutic and side effects.

In summary, our data demonstrated that JTP-117968, a novel SGRM that maintained the partial TR activity while remarkably reducing the TA activity, exhibited significant inhibitory effect on the TNFα production induced by LPS challenge in a mouse model. Notably, JTP-117968 also suppressed the mouse CIA development, comparably with prednisolone and PF-802, which is the active form of fosdagrocorat that has been developed clinically as an orally available SGRM. Strikingly, the effect of JTP-117968 on the femoral BMD in mice was much lower than those of prednisolone and PF-802. Therefore, JTP-117968 has attractive potential as a new therapeutic option against inflammatory and autoimmune diseases, such as rheumatoid arthritis, with minimized side effects compared to classic glucocorticoids.

Acknowledgements

We would like to thank Yuki Bessho, Shohei Misaki and Takeshi Wakashima (Japan Tobacco Inc.) for their technical assistance.

This study was funded by Japan Tobacco Inc. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Baiula, M., Spampinato, S., 2014. Mapracorat, a novel non-steroidal selective glucocorticoid receptor agonist for the treatment of allergic conjunctivitis. Inflamm Allergy Drug Targets 13, 289-298.

Barnes, P.J., 1998. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin. Sci. (Lond.) 94, 557-572.

Barnes, P.J., 2006. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. Br. J. Pharmacol. 148, 245-254.

Brandish, P.E., et al., 2014. The preclinical efficacy, selectivity and pharmacologic profile of MK-5932, an insulin-sparing selective glucocorticoid receptor modulator. Eur. J. Pharmacol. 724, 102-111.

Brown, M.N., et al., 2019. Efficacy and safety of AZD7594, an inhaled non-steroidal selective glucocorticoid receptor modulator, in patients with asthma: a phase 2a randomized, double blind, placebo-controlled crossover trial. Respir. Res. 20, 37.

Bungard, C.J., et al., 2011. Discovery of selective glucocorticoid receptor modulator MK-5932. Bioorg. Med. Chem. 19, 7374-7386.

Buttgereit, F., et al., 2019. Fosdagrocorat (PF-04171327) versus prednisone or placebo in rheumatoid arthritis: a randomised, double-blind, multicentre, phase IIb study. RMD Open 5, e000889.

Frey, F.J., et al., 2004. Glucocorticoid-mediated mineralocorticoid receptor activation and hypertension. Curr. Opin. Nephrol. Hypertens. 13, 451-458.

Hegelund Myrbäck, T., et al., 2020. Effects of a selective glucocorticoid receptor modulator (AZD9567) versus prednisolone in healthy volunteers: two phase 1, single-blind, randomised controlled trials. The Lancet Rheumatology 2, e31-e41.

Higuchi, Y., et al., 2006. Intravenous administration of mannosylated cationic liposome/NFkappaB decoy complexes effectively prevent LPS-induced cytokine production in a murine liver failure model. FEBS Lett. 580, 3706-3714.

Hu, X., et al., 2011. The antagonists but not partial agonists of glucocorticoid receptor ligands show substantial side effect dissociation. Endocrinology 152, 3123-3134.

Hudson, W.H., et al., 2013. The structural basis of direct glucocorticoid-mediated transrepression. Nat. Struct. Mol. Biol. 20, 53-58.

Jantzen, H.M., et al., 1987. Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. Cell 49, 29-38.

Johannesdottir, S.A., et al., 2013. Use of glucocorticoids and risk of venous thromboembolism: a nationwide population-based case-control study. JAMA internal medicine 173, 743-752.

Kurimoto, T., et al., 2017. JTP-117968, a novel selective glucocorticoid receptor modulator, exhibits improved transrepression/transactivation dissociation. Eur. J. Pharmacol. 803, 179-186.

Kwon, O.G., et al., 2014. The Effects of Platycodin D, a Saponin Purified from Platycodi Radix, on Collagen-Induced DBA/1J Mouse Rheumatoid Arthritis. Evid. Based Complement. Alternat. Med.

2014, 954508.

Lopez, F.J., et al., 2008. LGD-5552, an antiinflammatory glucocorticoid receptor ligand with reduced side effects, in vivo. Endocrinology 149, 2080-2089.

Madan, B., et al., 2012. SB1578, a novel inhibitor of JAK2, FLT3, and c-Fms for the treatment of rheumatoid arthritis. J. Immunol. 189, 4123-4134.

Marenzana, M., et al., 2011. Sclerostin antibody treatment enhances bone strength but does not prevent growth retardation in young mice treated with dexamethasone. Arthritis Rheum. 63, 2385-2395.

Mihara, K., et al., 2008. A potent and selective p38 inhibitor protects against bone damage in murine collagen-induced arthritis: a comparison with neutralization of mouse TNFalpha. Br. J. Pharmacol. 154, 153-164.

Miner, J.N., et al., 2007. Antiinflammatory glucocorticoid receptor ligand with reduced side effects exhibits an altered protein-protein interaction profile. Proc. Natl. Acad. Sci. U. S. A. 104, 19244-19249.

Moreno, J.J., 1997. Antiflammins: endogenous nonapeptides with regulatory effect on inflammation. Gen. Pharmacol. 28, 23-26.

Ohnaka, K., et al., 2004. Glucocorticoid enhances the expression of dickkopf-1 in human osteoblasts: novel mechanism of glucocorticoid-induced osteoporosis. Biochem. Biophys. Res. Commun. 318, 259-264.

Reuter, K.C., et al., 2012. Selective non-steroidal glucocorticoid receptor agonists attenuate

inflammation but do not impair intestinal epithelial cell restitution in vitro. PLoS One 7, e29756.

Sarett, L.H., 1946. Partial synthesis of pregnene-4-triol-17(beta), 20(beta), 21-dione-3,11 and pregnene-4-diol-17(beta), 21-trione-3,11,20 monoacetate. J. Biol. Chem. 162, 601-631.

Schacke, H., et al., 2002. Mechanisms involved in the side effects of glucocorticoids. Pharmacol. Ther. 96, 23-43.

Schacke, H., et al., 2004. Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. Proc. Natl. Acad. Sci. U. S. A. 101, 227-232.

Schacke, H., et al., 2009. Characterization of ZK 245186, a novel, selective glucocorticoid receptor agonist for the topical treatment of inflammatory skin diseases. Br. J. Pharmacol. 158, 1088-1103.

Shipp, L.E., et al., 2010. Transcriptional regulation of human dual specificity protein phosphatase 1 (DUSP1) gene by glucocorticoids. PLoS One 5, e13754.

Sohn, K.C., et al., 2013. Effects of Calcium Gluconate, a Water Soluble Calcium Salt on the Collagen-Induced DBA/1J Mice Rheumatoid Arthritis. Biomol. Ther. (Seoul) 21, 290-298.

Stock, T., et al., 2009. Evaluation of safety, pharmacokinetics and pharmacodynamics of a selective glucocorticoid receptor modulator (SGRM) in healthy volunteers, The 2009 ACR/ARHP Annual Scientific Meeting. Arthritis Rheum, Philadelphia, PA, p. 420.

Tammara, B., et al., 2013. Pharmacokinetic and pharmacodynamic characterization of a dissociated

agonist of glucocorticoid receptor, following Multiple Dose Administration In Healthy Japanese Adult Subjects., 2013 ACR/ARHP Annual Meeting. Arthritis Rheum, San Diego, CA, p. 2361.

Tamura, Y., et al., 2004. Glucocorticoid-induced osteoporosis. Biomed. Pharmacother. 58, 500-504.

Thiele, S., et al., 2012. Selective glucocorticoid receptor modulation maintains bone mineral density in mice. J. Bone Miner. Res. 27, 2242-2250.

van der Goes, M.C., et al., 2010. Patient and rheumatologist perspectives on glucocorticoids: an exercise to improve the implementation of the European League Against Rheumatism (EULAR) recommendations on the management of systemic glucocorticoid therapy in rheumatic diseases. Ann. Rheum. Dis. 69, 1015-1021.

van Lierop, M.J., et al., 2012. Org 214007-0: a novel non-steroidal selective glucocorticoid receptor modulator with full anti-inflammatory properties and improved therapeutic index. PLoS One 7, e48385.

van Staa, T.P., et al., 2002. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. Osteoporos. Int. 13, 777-787.

Wang, F.S., et al., 2008. Modulation of Dickkopf-1 attenuates glucocorticoid induction of osteoblast apoptosis, adipocytic differentiation, and bone mass loss. Endocrinology 149, 1793-1801.

Yamaguchi, T., et al., 2012. Suppressive effect of an orally active MEK1/2 inhibitor in two different animal models for rheumatoid arthritis: a comparison with leflunomide. Inflamm. Res. 61, 445-454.

Zhang, J.Z., et al., 2009. BOL-303242-X, a novel selective glucocorticoid receptor agonist, with full anti-inflammatory properties in human ocular cells. Mol. Vis. 15, 2606-2616.

Figure Captions

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

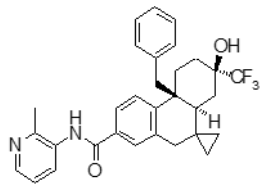
Fig. 2. Effect of JTP-117968 in lipopolysaccharide (LPS) challenge model mice. Female BALB/c mice were dosed orally with vehicle, JTP-117968, PF-802, or prednisolone 1 hour before a challenge with an intravenous dose of LPS. Blood samples were collected from mice 90 min after LPS challenge and plasma levels of Tumor Necrosis Factor α (TNFα) were determined by Mouse TNFα Immunoassay. Data are presented as means ± standard deviation (n=3-7). $^{‡‡}P<0.01$ and $^{‡‡‡}P<0.001$ versus vehicle using Student's *t*-test. $^{§§}P<0.01$ versus vehicle using Steel's test.

Fig. 3. Effect of JTP-117968 on collagen-induced arthritis in mice. Test compounds were administered orally once daily from the second immunization with type II collagen (day 22). (A) Shows the time-course changes in arthritic score and (B) shows arthritic score on day 36. Data are presented as means ± standard deviation (n=10). $^{†††}P<0.001$ versus sham using Welch's *t*-test. $^{‡‡‡}P<0.001$ versus vehicle using Student's *t*-test. $^{$$$}P<0.001$ versus vehicle using Welch's *t*-test. $^{§}P<0.05$, $^{§§§}P<0.001$ versus vehicle using Steel's test. Mean arthritis score was determined in a blind fashion.

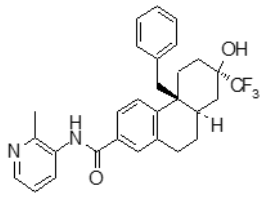
Fig. 4. Effect of JTP-117968 on the change of relative spleen weight in collagen-induced arthritis (CIA) mice. The relative weight of spleens in CIA mice was calculated using absolute weight of spleens and body weight at sacrifice on day 36. Data are presented as means ± standard deviation (n=10). $^{\#\#\#}P<0.001$ versus sham using Student's *t*-test. $^{‡‡‡}P<0.001$ versus vehicle using Student's *t*-test. $^{\$\$\$}P<0.001$ versus vehicle using Welch's *t*-test. $^{***}P<0.001$ versus vehicle using Dunnett's test.

Fig. 5. Effect of JTP-117968 on bone mineral density (BMD) in normal BALB/c mice after 28 days of repeated oral dosing. Test compounds were administered to female BALB/c mice orally once daily for 28 days. On the day following the last administration, the left femurs were excised and cleaned of excess soft tissue. The BMD of each femur was measured by peripheral quantitative computed tomography. Data are presented as means ± standard deviation (n=10). $^{***}P<0.001$ versus vehicle using Dunnett's test. $P=0.091$ and P=0.053 versus vehicle also using Dunnett's test.

Fig. 6. Effect of JTP-117968 on Dickkopf-1 (Dkk-1) mRNA expression in primary human osteoblasts. Induction of Dkk-1 mRNA in human primary osteoblasts was determined for JTP-117968 and prednisolone (A). Effect of PF-802 on Dkk-1 mRNA expression in primary human osteoblasts (B). Efficacies are shown relative to the response to 0.1% Dimethyl Sulfoxide (DMSO). Data are representative of the means ± standard deviation (n=3). Pred: prednisolone.



JTP-117968



PF-802

Fig. 1

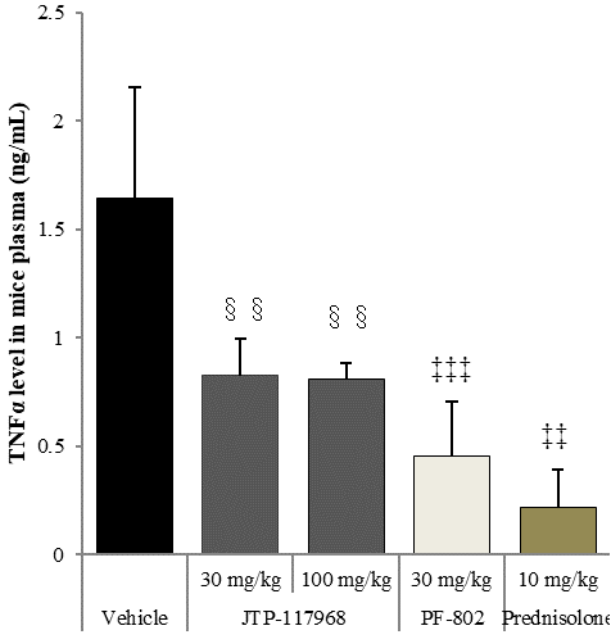

TNFα level in mice plasma (ng/mL)
2.5
2
1.5
1
0.5
0
§ §
§ §
‡‡‡
‡‡
30 mg/kg
100 mg/kg
30 mg/kg
10 mg/kg
Vehicle
JTP-117968
PF-802
Prednisolone

Fig. 2

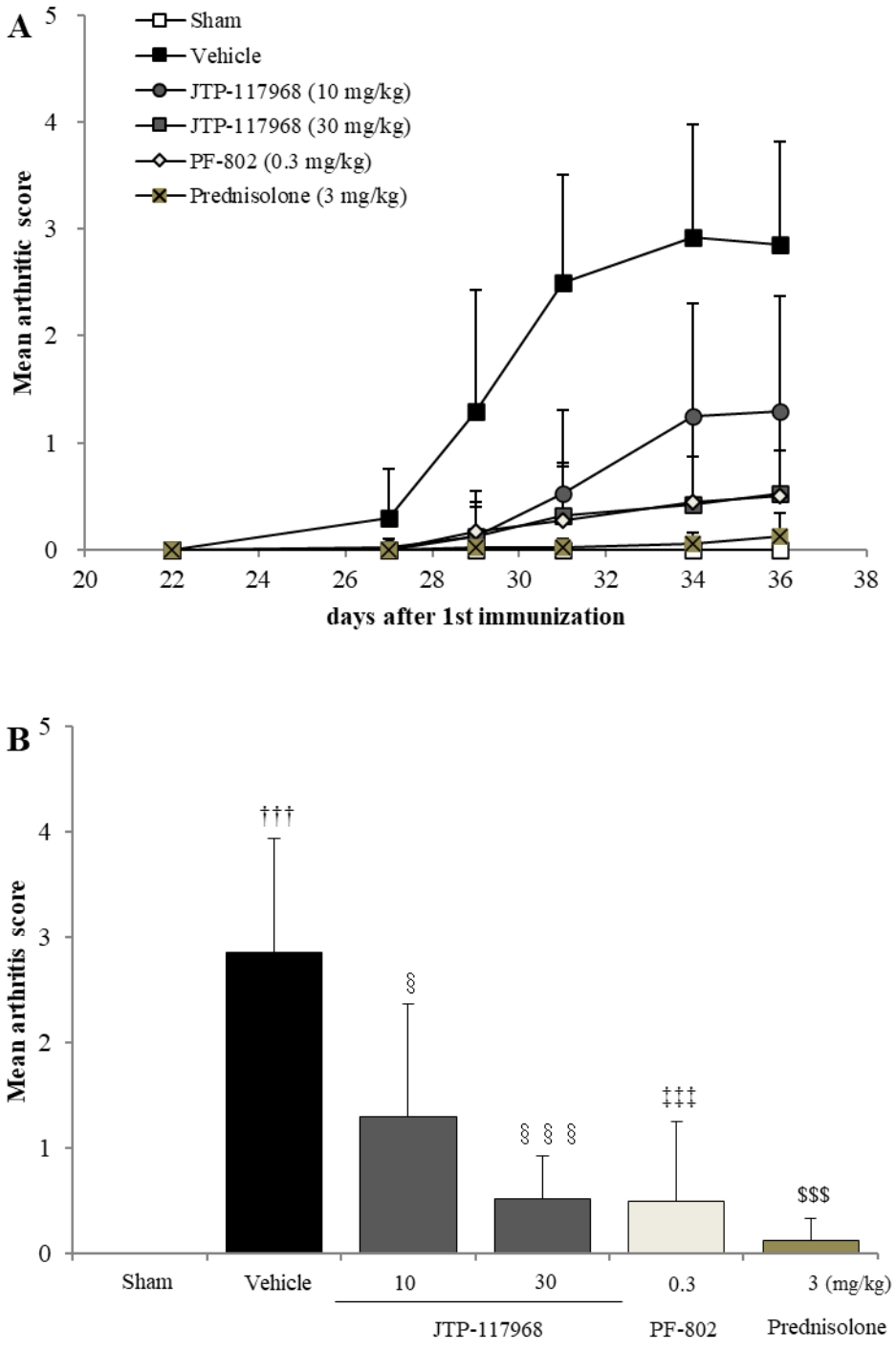

A
Sham
Vehicle
JTP-117968 (10 mg/kg)
JTP-117968 (30 mg/kg)
PF-802 (0.3 mg/kg)
Prednisolone (3 mg/kg)
Mean arthritic score
days after 1st immunization
B
Mean arthritis score
†††
§
§ § §
‡‡‡
$$$
Sham
Vehicle
10
30
0.3
3 (mg/kg)
JTP-117968
PF-802
Prednisolone

Fig. 3

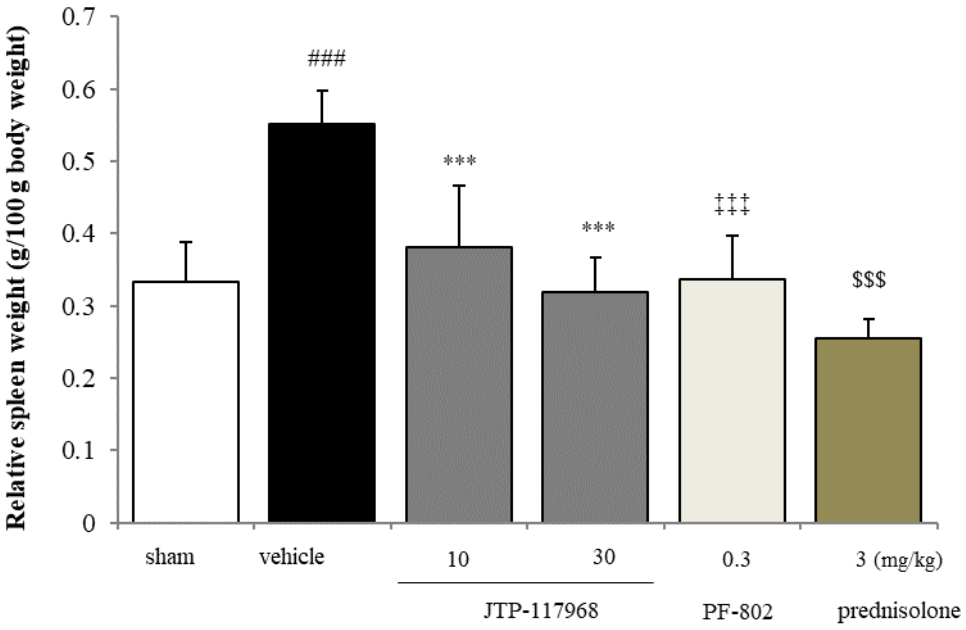

Relative spleen weight (g/100 g body weight)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
###
***
***
‡‡‡
$$$
sham
vehicle
10
30
0.3
3 (mg/kg)
JTP-117968
PF-802
prednisolone

Fig. 4

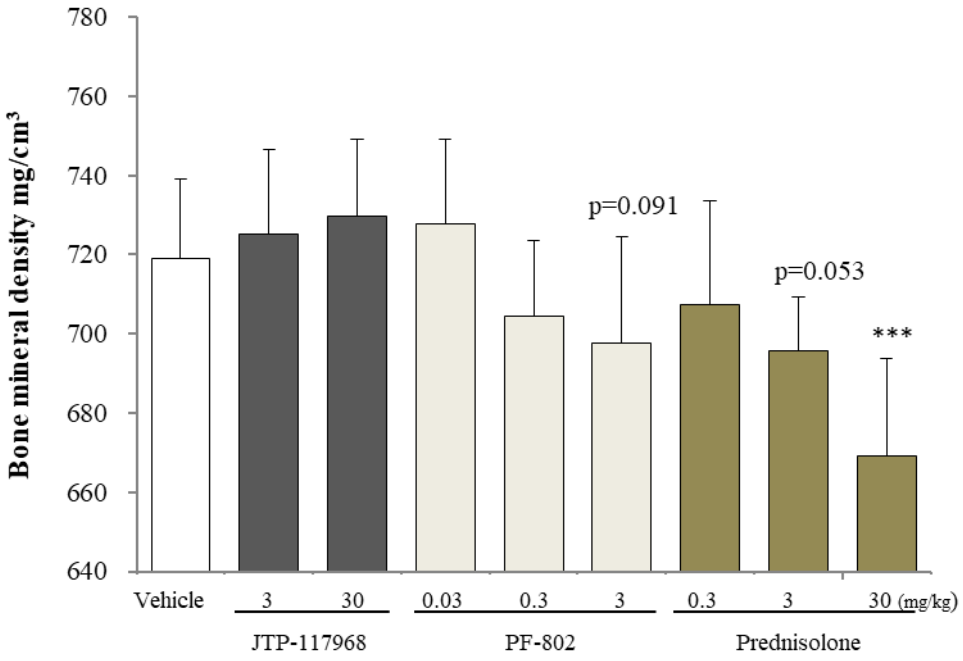

Bone mineral density mg/cm³
780
760
740
720
700
680
660
640
p=0.091
p=0.053
***
Vehicle
3
30
0.03
0.3
3
0.3
3
30 (mg/kg)
JTP-117968
PF-802
Prednisolone

Fig. 5

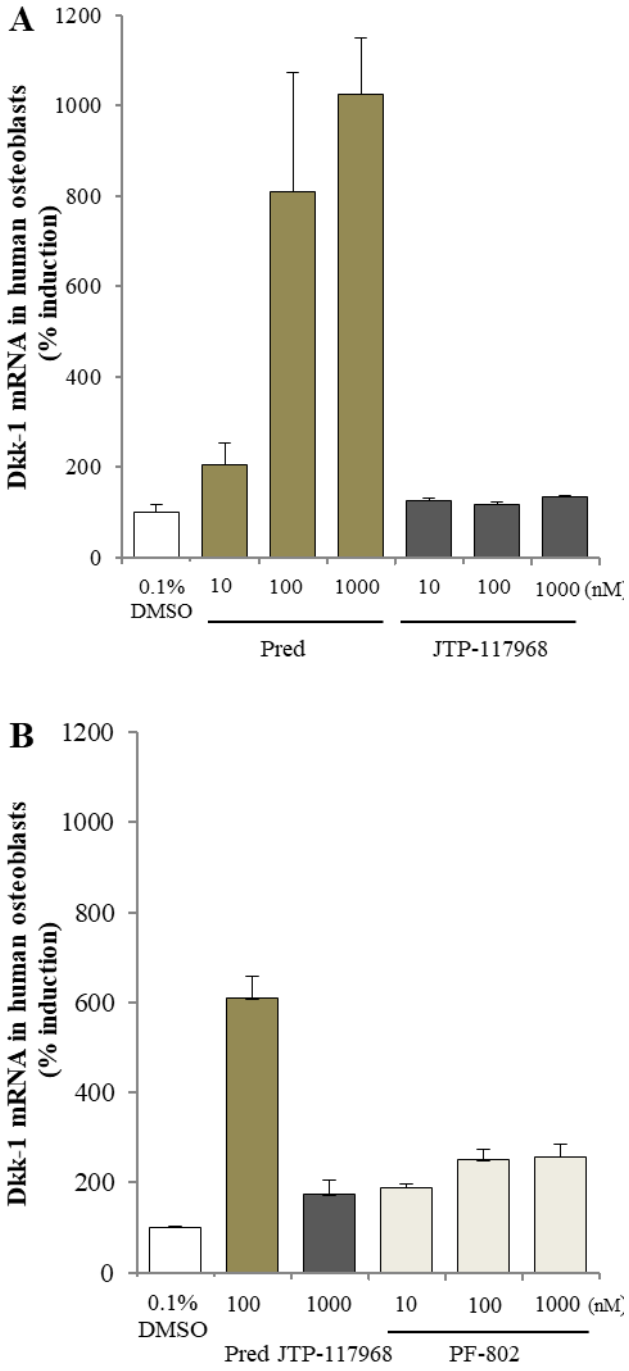

A
Dkk-1 mRNA in human osteoblasts (% induction)
1200
1000
800
600
400
200
0
0.1% DMSO
10
100
1000
10
100
1000 (nM)
Pred
JTP-117968
B
Dkk-1 mRNA in human osteoblasts (% induction)
1200
1000
800
600
400
200
0
0.1% DMSO
100
1000
10
100
1000 (nM)
Pred JTP-117968
PF-802

Fig. 6