Ginsenosides as selective glucocorticoid drugs: agonists, antagonists, and prodrugs

Halima, M.

Chapter 8

Tramadol/paracetamol treatment attenuates the development of collagen antibody-induced arthritis and interferes with prednisolone treatment in mice

Sanne Kroos*[1], Mahmoud Halima*[2], Jan Kroon[3], Diane van der Woude[1], Onno C. Meijer[3], Maarten D. van de Wal[4], Peternella S. Verhave[5], Marcel J.M. Schaaf[2], René E.M. Toes[1], Arieke S.B. Kampstra[1].

[1]Department of Rheumatology, Leiden University Medical Center, The Netherlands
[2]Institute of Biology Leiden, Leiden University, The Netherlands
[3]Division of Endocrinology, Department of Medicine, Leiden University Medical Center, The Netherlands
[4]Central Animal Facility, Leiden University Medical Center, The Netherlands
[5]Animal Welfare Body Leiden, Leiden University Medical Center and Leiden University, The Netherlands

* These authors contributed equally

Lab Anim. 2023 Apr 17;236772231166029.

Abstract

The collagen antibody-induced arthritis (CAIA) model is highly effective in inducing arthritis, making it an attractive model for screening therapeutic compounds such as glucocorticoids (GCs). The severity of discomfort in this model makes it desirable to administer analgesics, but it is a prerequisite that these do not interfere with the model or tested therapeutics. In the present study, we studied the effect of 1 mg/mL tramadol and 3.5 mg/mL paracetamol (TP) on CAIA in male BALB/cAnNCrl mice and the possible interference of TP analgesia with the activity of the GC drug prednisolone (Pred). Our results showed that TP abolished the Pred-induced amelioration of CAIA, as well as several other Pred-induced effects, such as the reduction in thymus weight and the increase in insulin level. This most likely results from the effects of TP on the hepatic metabolism of this drug, since it strongly increased the *Cyp3a11* expression in the liver. Altogether, we conclude that TP analgesia is not suitable for the CAIA model in male BALB/cAnNCrl mice, in particular when evaluating the effects of GCs such as Pred.

1. Introduction

In the collagen antibody-induced arthritis (CAIA) model, arthritis is induced by the injection of a monoclonal antibody cocktail directed against type II collagen followed by an injection with LPS to enhance the arthritis synchronization and severity[1, 2]. In contrast to the collagen-induced arthritis (CIA) model, in which a cross-reactive immune response against type II collagen is elicited through injection with chicken or bovine type II collagen[3] resulting in arthritis onset after 4 weeks, arthritis in the CAIA model becomes readily visible after 24-48 hours following LPS injection[1, 4]. This rapid and effective arthritis onset makes it an attractive model to screen anti-inflammatory therapeutics, such as glucocorticoids (GCs).

GCs are very potent anti-inflammatory drugs used to treat a variety of diseases including allergies, chronic inflammatory diseases and autoimmune diseases like Rheumatoid Arthritis. After entering cells, GCs bind to the glucocorticoid receptor (GR) which acts as a transcription factor modulating the expression of several GR-target genes, leading to systemic immunosuppression[5]. However, unwanted regulation of GR-target genes leads to a variety of GC-induced side effects[6]. For this reason, novel anti-inflammatory GCs with fewer or no side effects are desired, of which the efficacy can be tested in the CAIA model.

The CAIA model is suitable to study the effects of anti-inflammatory drugs on arthritis, nevertheless the severity of discomfort induced by this model makes it desirable to reduce pain and improve the welfare of the mice. Ideally, analgesics are administered to mice undergoing CAIA, provided these compounds should not interact with the induction of the arthritis or the functionality of the therapeutics under investigation. For example, NSAIDs are unsuitable due to their anti-inflammatory properties. Additionally, buprenorphine should not be used since it has been shown to alter the expression of inflammatory markers in the CIA model and would therefore be a confounding factor when studying anti-inflammatory GCs[7]. In the study described here, tramadol and paracetamol were selected since no clearly defined anti-inflammatory effects of these compounds had been reported. Paracetamol alone was presumed to be not sufficiently effective and was therefore co-applied with the opioid tramadol to enable multimodal analgesia[8, 9].

In the present study, we have determined whether the combination of paracetamol and tramadol (TP) interferes with the CAIA model when testing the anti-inflammatory effects of the GC Pred. For this purpose, we induced CAIA in a total of 16 male BALB/cAnNCrl mice, divided into four groups: two groups were treated with Pred, of which one group received TP through the drinking water. To exclude effects of the dissolvent, two groups were treated with the dissolvent only (referred to as Vehicle (Veh)) of which one group received TP. It was observed that TP influences disease severity and, additionally, impeded with the Pred-induced alleviation of the arthritis. Moreover, GC-related side effects were influenced by TP administration, including endogenous corticosterone and insulin levels, thymus and adrenal gland weight and expression of multiple GR-target genes. Our observations indicate that TP analgesia can influence disease severity and interferes with several Pred-induced (side) effects, making TP incompatible with testing Pred and possibly also other GCs in the CAIA model.

2. Methods

2.1. Mice and husbandry

Male BALB/cAnNCrl mice have high CAIA susceptibility and were therefore selected for this study[1]. The mice were purchased from Charles River and upon arrival, and sixteen mice were divided into four treatment groups: two Pred-treated groups of which one would receive analgesia (Pred + TP), and two Veh-treated groups of which one would receive analgesia (Veh + TP), n=4 per group. Mice receiving only the vehicle were included to determine the side effects brought about by the Pred treatment in order to assess the impact of TP administration. The sample size was calculated using the method for independent T-tests, with an alpha (type 1 error) of 2.5% and a power of 0.8, based on an anticipated normal distribution of arthritis scores.

All mice were housed in individually ventilated cages under specific pathogen-free conditions at the animal facility of Leiden University Medical Centre (LUMC). Each cage housed four mice and contained special bedding material (Enrich-n'Pure, The Andersons), nesting material (Nesting Cup, Carfil) and a GLP fun tunnel (LBS Biotech) as enrichment. The mice receive pelleted food *ad libitum* (RM3 diet, irradiated 9kGy, SDS diets) as well as DietGel recovery cups (ClearH2O) to ensure sufficient hydration and nutrient intake. The light cycle was set to mimic sunrise at 06.30 AM and sunset at 6.30 PM. The temperature was kept at an average of 21°C (min: 20°C, max: 22°C). Relative humidity was set at an average of 50% (min: 40%, max: 60%).

2.2. Ethical statement

The experiment was reviewed by the Animal Welfare Body of the LUMC and executed under a license granted by the Central Authority for Scientific Procedures on Animals under license number AVD11600202115391, in accordance with the Dutch Act on Animal Experimentation and EU Directive 2010/63/EU.

2.3. Arthritis induction and scoring

Mice were approximately 8 weeks of age upon arrival at the animal facility. After 1 week of acclimatization, on day (d) 1 of the study, mice were restrained in a tail-first restrainer and received 1.5mg of CAIA-cocktail (Chondrex) intravenously via the tail vein. On d4, the mice

were scruff restrained and received 50µg LPS (Chondrex) though intraperitoneal injection. On d5, the mice were given DietGel® Recovery (Clear H_2O) to support their recovery from the LPS injection. Arthritis was scored daily from d6 until d15 as described previously[10]. Each swollen or red phalanx was assigned 0.5 point and each swollen or red knuckle was given 1 point. In addition, each swollen or red paw padding was given 1 point and each swollen or red ankle or wrist was given 5 points. Altogether, the scoring results in a maximum of 15 points per paw, or 60 points per mouse[10]. Nevertheless, a maximum of a total of 45 points per mouse will be allowed (see 'Humane endpoints'). Arthritis scores were always determined by the same person to reduce variation in score interpretation. The person determining arthritis scores was always blinded for treatment allocation. Treatment itself and data analysis was performed in a non-blinded manner.

2.4. Treatments and handlings

Pred- and Veh-treated groups were divided over TP-treated cages and non-TP treated cages to minimize cage effects. TP was administered from d6 onwards, via drinking water containing 1mg/mL tramadol hydrochloride (Merck) and 3.5mg/mL paracetamol (Dafalgan), both mixed in the same drinking bottle. The sweet taste of paracetamol-containing Dafalgan syrup ensured the mice would still drink their water despite the bitter taste of tramadol. Based on the average consumption of an individual BALB/cAnNCrl mouse of 6mL/30g body weight per 24 hours[11], mice weighing 18-24 grams drink 3.6-4.8 mL of water containing 12.6-16.8 mg paracetamol and 3.6-4.8 mg tramadol daily. Like many corticosteroids, Pred (Sigma-Aldrich) is poorly soluble in water and was therefore reconstituted in 10% EtOH and administered at a concentration of 25mg/kg body weight. To exclude effects of the dissolvent, the Veh-control group received 10% EtOH only. Pred or Veh treatments were administered daily from d7 until d15 via intraperitoneal injection upon scruff restraining. Blood was collected by tail-vein sampling on d0 (75µl), d10 (75µl) and d14 (40µl) by restraining the mice in a tail-first restrainer. The d14 bleeding was performed after 6 hours of fasting (water was available), which is required for reliable determination of insulin serum levels. On d15, all mice were sacrificed by gradual exposure to carbon dioxide and blood was collected through heart puncture. Blood on d0 and d10 was collected in clotting activator tubes (Starstedt Microvette® CB300) resulting in serum, whereas blood on d14 was collected in sodium heparin capillaries (Carl Roth) resulting in plasma.

2.5. Humane endpoints

Humane endpoints were set for weight loss and arthritis score. Body weight was monitored by daily weighing of the mice. A total body weight loss of >20% in comparison to the day of LPS injection was set as a weight loss maximum, while allowing a 25% weight loss for no longer than one day. For arthritis score, a score of 45 was set as a maximum. This maximum score would mean the mouse has reached maximal arthritis in 3 paws or high arthritis in 4 paws, see 'arthritis induction and scoring'. Reaching an arthritis score of 45 would severely impair normal behavior (such as washing, eating, walking, sleeping) which is avoided by setting this humane endpoint. Reaching humane endpoints for weight loss or arthritis score would mean humanely sacrificing the animal by gradual exposure to carbon dioxide.

2.6. Body composition analyses

On d0 and d15, the body fat weight in grams was determined using the EchoMRI™-100H body composition analyzer[12]. The lean body weight was calculated by subtracting the body fat weight from the total body weight. On d15, after the mice were sacrificed, the thymus and left adrenal gland were collected and weighed.

2.7. Liver and muscle gene expression analyses

On d15, liver and quadriceps muscle tissue were collected in TRIzol (Invitrogen) and mRNA was isolated using the miRNeasy mini kit (Qiagen), according to the manufacturer's instructions. Samples were treated with DNAse using DNA-free™ (Ambion). The cDNA synthesis was performed using iScript (Bio-Rad) using 1µg RNA per sample. For qPCR, 10µM of forward and 10µM of reverse primer (see table 1 for sequences), 12.5µL of iQ SYBR Green Supermix (Bio-Rad), and 1µl cDNA were added to the qPCR reaction mixture. The qPCR reactions were performed on a MyiQ™ single-color real-time PCR-detection system (Bio-Rad) with denaturation for 3min at 95°C and 40 cycles of 15s at 95.5°C, 15s at 60°C and 30s at 72°C. Cycle threshold values (Ct values, i.e. the cycle numbers at which a threshold value of the fluorescence intensity was reached) were determined for each sample. The gene expression level for each sample was normalized using the expression of *β-actin*. The fold change per sample (compared to the respective control group) was calculated using the ΔΔCt method. In each experiment, four mice were used for each treatment group (except for the Veh + TP group, for which 3 mice were used). All reactions were performed in duplicates. The sequences of all qPCR primers are depicted in Table 1.

2.8. ELISAs

Plasma collected on d14 was used to determine insulin levels by using the ultra-sensitive mouse insulin ELISA kit (Crystal Chem), following the manufacturer's protocol[13]. Serum collected on d15 was used to measure corticosterone levels using the HS enzyme immunoassay kit (Immunodiagnostic Systems), following the manufacturer's protocol[14].

2.9. Statistical analyses

During the sample size calculations, we anticipated a normal distribution of the arthritis scores. Nevertheless, upon visual inspection of the data, the standards required for parametric statistical testing were not met. Therefore, the non-parametric Mann-Whitney U test was applied for all statistical analyses to determine significance of observed differences. To determine whether TP administration had an effect on Pred treatment in the CAIA model and side effects of Pred, significant differences were only determined between the Veh-treated group and the Pred-treated group, with or without TP. Important to note is that, with this study, we want to find out whether analgesia can be applied and would provide us with a similar extent in parameters required to evaluate whether alternatives to Pred are superior. Therefore, no analysis has been performed between the non-TP and TP-treated groups, except when the effect of TP on GR target-gene expression was evaluated.

All statistical analyses were performed using GraphPad Prism 9. Notion should be taken when interpreting the p-values since the Veh + TP-treated group included only 3 mice and therefore comparisons to this group cannot reach statistical significance.

3. Results

3.1. Health and welfare observations

Body weight was monitored daily throughout the course of the study. A 20% body weight loss was initially intended as a humane endpoint. However, as reported previously[15, 16], mice can temporarily lose up to 20-30% of their body weight following LPS injection. Indeed, weights remained stable until LPS injection on d4, after which a rapid decline was observed (figure 1 A and 1 B) and the mice were considerably less active. After 48 hours the mice should be re-gaining weight[15]. For this reason, a total body weight loss of >20% in comparison to the day of LPS injection was set as a weight loss maximum, while allowing a 25% weight loss for no longer than one day. Indeed, the overall state of most mice and the weight loss started to stabilize on d7, except for two mice. These mice crossed the 20% body weight loss border but showed the expected recovery the next day. These mice remained in the experiment as application of a "strict" 20% cut off would result in having to include more mice in the experiment to reach sufficient power.

Furthermore, lean body weights were determined (figure 1 C). Though not reaching statistical significance, Pred-treated mice tended to have lower total body weights and lean body weights compared to Veh-treated mice.

One mouse, assigned to the Veh + TP treatment group, was found deceased in its cage on d6. No unusual observations were made during internal and external inspection of the carcass and therefore the cause of death remained unknown (accidental LPS injection into organs was excluded since no visible inflammation of the gut and its surroundings was present). The mouse was excluded from the analysis resulting in only 3 mice in the Veh + TP group, whereas all other experimental groups contained 4 mice.

The mice started showing signs of arthritis on d6. Before d6, no visible swelling or redness was present yet, although the mice were not officially scored. As the first day after LPS injection led to the mice being notably lethargic, weight was the only parameter measured to minimize stress and associated additional discomfort. Interestingly, arthritis development remained limited to the hind paws in all mice throughout the study, and none of the mice reached the humane endpoint of an arthritis score of 45. In addition, no irritations as a result of the 10% EtOH i.p. (vehicle) injections were observed.

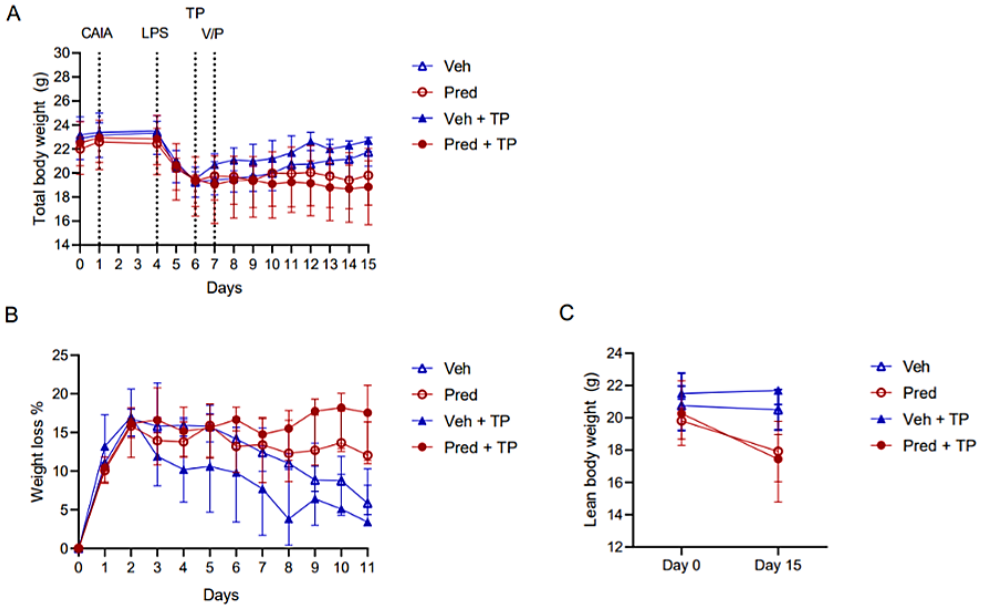

Figure 1. Body weight of the mice over the course of the study. *A. Total body weight in grams of the mice as measured each study day. B. Weight loss % in comparison to the weight on the day of LPS injection. C. Lean body weight in grams as calculated by subtracting body fat weight from the total body weight. Each line indicates the median values per group, interquartile ranges are depicted. CAIA: day of CAIA cocktail administration. LPS: day of LPS administration. TP: start of TP administration. V/P: start of daily Veh- or Pred treatment.*

3.2. TP alters arthritis development and reduces Pred efficacy

From d8 onwards, Veh-treated mice reached an arthritis score of approximately 15 which remained stable throughout the course of the experiment. As expected, Pred-treated mice showed rapidly decreasing arthritis scores after treatment initiation on d7, reaching scores of approximately 0 at d11 (figure 2 A). Interestingly, in mice receiving TP, the arthritis scores were modest and reached maximal values of approximately 10 at d8 (figure 2 B). Moreover, the therapeutic effect of Pred treatment was absent with TP administration. While there was a clear difference in arthritis scores between Veh- and Pred-treated mice, this difference was not observed between Veh + TP-treated mice and Pred + TP-treated mice (figure 2 C).

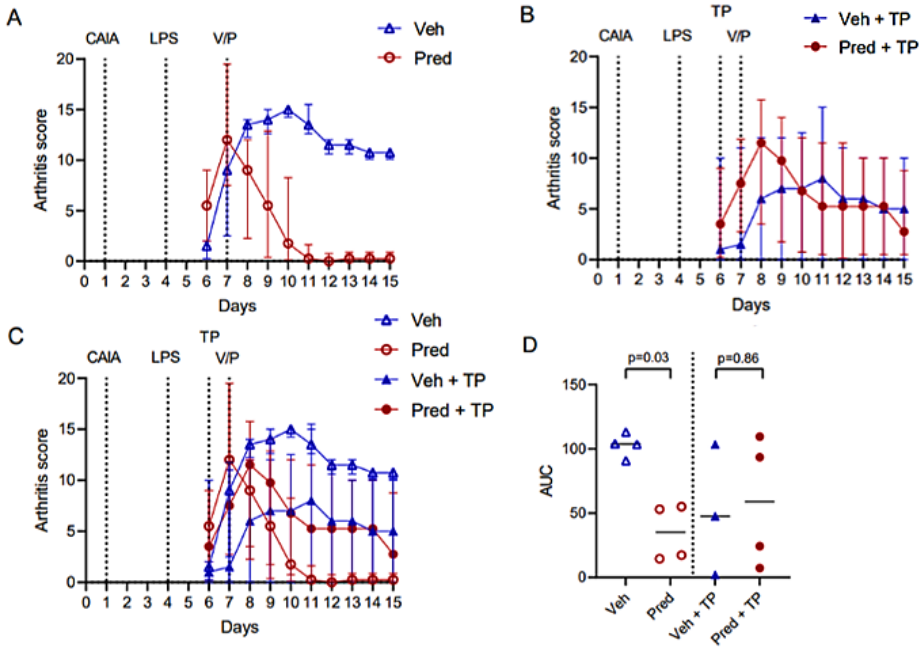

Figure 2. Arthritis scores of mice during the course of the study. *A. Arthritis scores of Veh- and Pred-treated mice. Each line indicates the median values per group, interquartile ranges are depicted. B. Arthritis scores of Veh + TP- and Pred + TP-treated mice. Each line indicates the median values per group, interquartile ranges are depicted. C. Arthritis scores of all treatment groups combined. Each line indicates the median values per group, interquartile ranges are depicted. All data resulted from the same experiment, but are separated in 2A and 2B for visualization purposes C. Area under the curve (AUC) values of arthritis scores. Individual values and medians are depicted. CAIA: day of CAIA cocktail administration. LPS: day of LPS administration. TP: start of TP administration. V/P: start of daily Veh- or Pred-treatment.*

3.3. TP influences Pred-induced effects as measured by metabolic parameters

In order to develop novel GCs with reduced side effects, proper determination of these side effects in an animal model is important. Therefore, when testing novel GC compounds, the chosen analgesics should not interfere with the therapeutic and/or potential side effects of the compounds under investigation. It is well established that GC treatment triggers thymocyte apoptosis[17] and adrenal gland atrophy[18]. We therefore analyzed the weights of the thymus and the adrenal glands of all mice, and the results showed that Pred treatment indeed decreased the thymus and adrenal gland weights (figures 3 A, 3 B). This GC effect on the thymus weight was absent in Pred + TP-treated mice (figure 3 A), but the effect on the adrenal gland weight was still present in Pred + TP-treated mice (figure 3 B).

Additionally, GCs are known to suppress corticosterone production through negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis[19]. We indeed observed a Pred-

induced reduction in corticosterone levels, also in Pred + TP-treated mice, indicating TP does not alter this effect (figure 3 C). Furthermore, GC therapy is known to increase insulin production[20], and we observed an increased insulin level in the Pred-treated mice. However, this effect was absent in Pred + TP-treated mice, indicating an inhibitory effect of TP on the Pred-induced increase in the insulin level (figure 3 D).

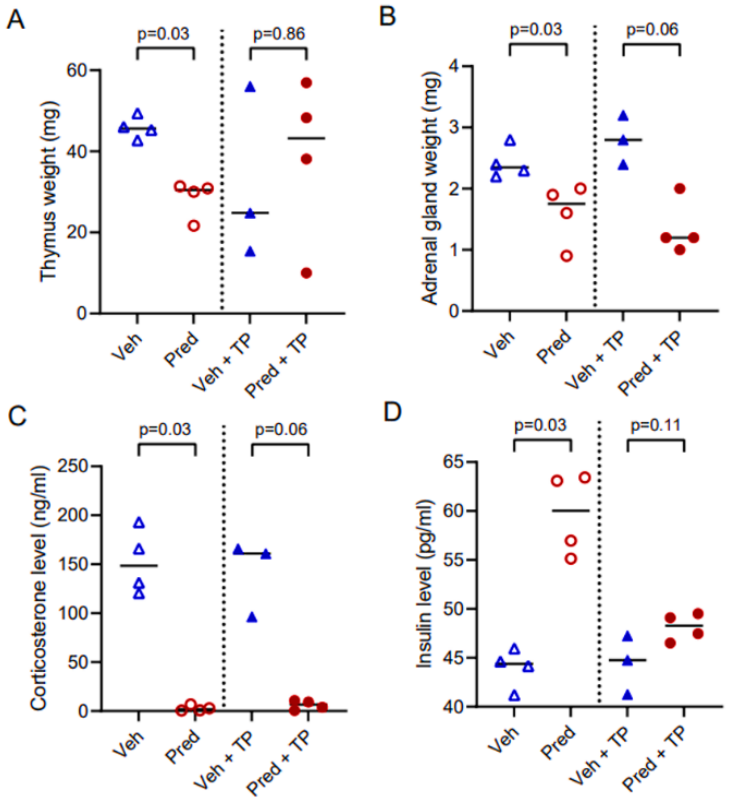

Figure 3. TP affects Pred-induced effects*. A. Thymus weight in milligrams as determined after thymus isolation on d15. B. Adrenal gland weight in milligrams as determined after adrenal gland isolation on d15. C. Corticosterone levels in serum collected on d15. D. Insulin levels in plasma collected on d14 after a 6-hour fasting period from 07.00 AM until 13.00 PM. Individual values and medians are depicted.*

3.4. TP influences Pred-induced effects on gene expression in liver and muscle tissue

In addition to metabolic parameters, proxies for GC-induced side effects can be measured at the level of gene expression of several well-established genes involved in the induction of side effects. To this end, analysis was performed on the expression of four GR-target genes, known to be implicated in muscle atrophy upon GC exposure: *Redd1*, *Foxo3*, *Gs* and *Klf15*[21]. All four analyzed genes were upregulated in muscle tissue upon Pred treatment. However, in muscle tissue isolated from Pred + TP-treated mice, a significantly lower induction of the expression of these genes was observed (figure 4 A). Likewise, we determined the expression of GR-target genes in liver tissue including classical target genes like *Fkbp5* and *Gilz* as well as genes involved in GC-induced hyperglycemia: *Pck1* and *G6pd*[22, 23]. *Fkbp5, Gilz and Pck1* were upregulated in Pred-treated mice, but showed lower induction of the expression levels in Pred + TP-treated mice (figure 4 B). Intriguingly, TP-induced upregulation of *Cyp3a11*, encoding a liver enzyme involved in Pred metabolism[24], was found in both the Veh + TP- treated and the Pred + TP-treated group (figure 4 C).

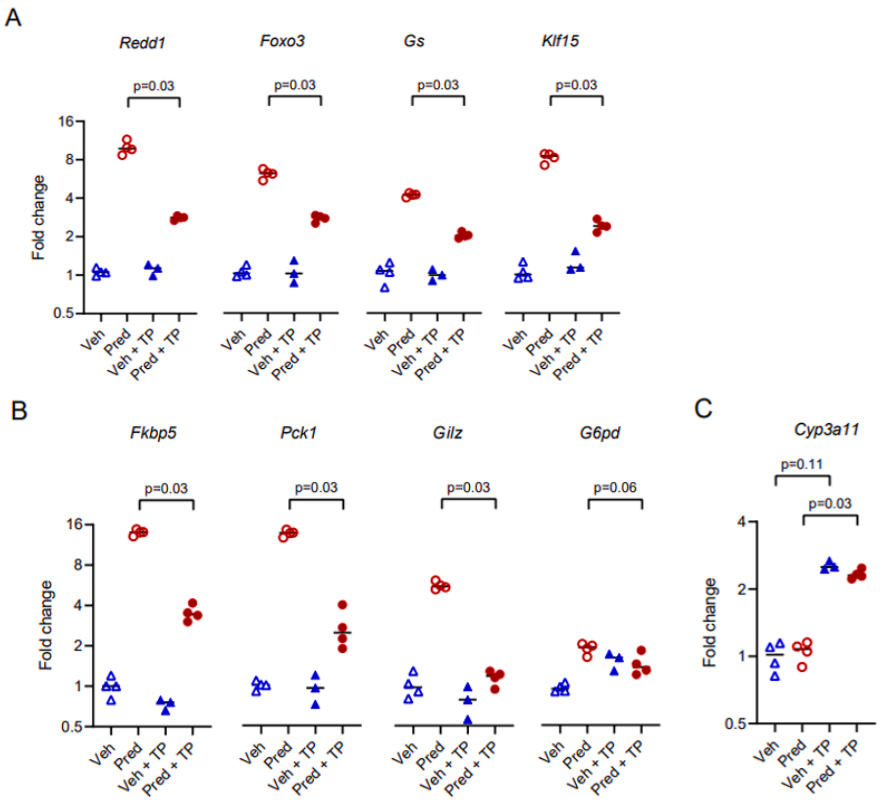

Figure 4. TP effects on the expression of GR-target genes*. A. Expression of GR-target genes in quadriceps muscle tissue. B. Expression of GR-target genes in liver tissue. C. Expression of Cyp3a11 in liver tissue. Individual values and medians are depicted.*

4. Discussion

The CAIA model is a very efficient mouse model for arthritis that is widely used for testing the efficacy of anti-inflammatory compounds such as GCs. However, the model induces a high degree of discomfort for the mice, so it would be desirable to decrease the severity of this model by treating the mice with analgesics, such as TP. We dedicated the present study to investigate whether TP influences the CAIA model and whether it has any interactions with the actions of Pred in this model. Since side effects induced by novel GCs are important parameters for determining superiority over conventional GCs, these should preferably not be influenced by TP.

The results described here show that TP administration perceivably diminishes the severity of arthritis as measured by visible swelling and redness of the joints. In the TP-treated mice, overall arthritis scores remained lower compared to the non-TP-treated mice. Whereas this is beneficial for the mice, it is problematic when using the CAIA model since this indicates TP introduces a bias when evaluating anti-arthritic drugs. No clear-defined anti-inflammatory properties of tramadol or paracetamol have previously been described. However, part of the underlying mechanism may be paracetamol-induced inhibition of cyclooxygenase-2 (COX-2), an enzyme that has been shown to be essential for developing collagen-induced arthritis[25]. Nonetheless, since there were no experimental groups receiving only paracetamol or tramadol, possible anti-inflammatory effects of tramadol alone cannot be dismissed and this should be investigated before using tramadol only in the CAIA model. Additionally, it has been described previously that tramadol can affect body weight and food/water intake during the first days after the start of treatment[26]. Such effects were not observed for body weight in the TP-treated mice, possibly due to the dominating effects of LPS injection. Even though tramadol-induced effects on body weight and nutrient intake has not been found to form an aggravated issue during this pilot study, it should be taken into account when considering tramadol as analgesic.

Our results show that TP interacts with Pred treatment as well. At the level of arthritis scores, this is indicated by the abrogated therapeutic window between Pred + TP- and Veh + TP-treated mice; Pred treatment seemed less efficient in terms of reducing arthritis when co-applied with TP. Additionally, we examined several well-established side effects induced by Pred treatment and observed TP interference. TP affected Pred-induced changes in both thymus weight and insulin level, as well as expression of genes involved in muscle atrophy and induction of hyperglycemia. Since these parameters are influenced by TP, TP analgesia resembles a confounding factor and should therefore not be used when investigating novel GCs and their (in)ability to induce side effects in the CAIA model. Furthermore, we observed a TP-induced upregulation of *Cyp3a11*, the mouse homologue of human *CYP3A4*, which encodes for an enzyme that has been shown to be involved in Pred metabolism[24]. This finding offers a possible explanation for the modulation of Pred-induced effects by TP, since it suggests that this treatment may increase the metabolism and clearance of Pred and thereby decrease its bioavailability. To our knowledge, no effects of tramadol or paracetamol on the Pred efficacy or metabolism have been described before so it remains unknown whether this effect on Pred metabolism is caused by tramadol or paracetamol.

Since our study was designed with the primary objective of determining whether TP can be used as an analgesic when testing novel GCs in the CAIA model, our study has a few

limitations. The arthritis scores of the TP-treated groups showed a remarkably high degree of variation. Since TP was administered through drinking water in the absence of water intake monitoring, mice could have consumed a variable drug intake possibly explaining the relatively high level of variation in several parameters. Additionally, this study did not include behavioral assessments to determine TP efficacy, so adequacy of TP on ameliorating mouse well-being cannot be confirmed. It is important to consider that only one dosage of TP and Pred was analyzed in a single mouse strain and gender. Ideally, a similar study should be performed in mice with a different genetic background and varying TP and /or Pred dosages.

An extensive review on the application of refinement during animal experiments used for arthritis research coined the term 'rescue analgesia', analgesics applied when the animal shows visible signs of pain[27]. These signs would be assessed daily during the experiment, leading to variable administration of analgesics per mouse. The application of TP for this particular mode of acute pain amelioration would imply that the variation that we have observed in arthritis scores could possibly be enlarged as well as the impact TP has shown on Pred side effects. Nevertheless, the application of an analgesic that does not interfere with the CAIA model or Pred activity would be a recommended candidate for rescue analgesia, although such application should be extensively investigated beforehand.

Based on the results presented here, we expect TP administration as analgesia to be a confounding factor in the CAIA model in male BALB/cAnNCrl mice, since there is a visual trend of arthritis scores being lower when using TP. Moreover, TP analgesia should not be used when testing GCs such as Pred, since TP may interfere with the activity of these compounds. These data provide valuable insight in the possible confounding factors influencing research results when refining animal models.

5. Supplementary materials

Table 1. **Sequence of primers for the qPCR analysis.**

Gene	Sequence
Redde1 – forward	GTCCAAAGCCTCAGAGTCGTTC
Redde1 – reverse	AGCCTTTCTCATTGGCACTGTC
Foxo3 – forward	AGCATTCAACGCCAGGTTC
Foxo3 – reverse	CGAGTCTGTCAGTTCAATACCAA
Gs – forward	CATCACCCTGGTACAACTCTT
Gs – reverse	CGGCAACTAAACTCAGAAAAC
Klf15 – forward	TCTTCATGCCACCATCATGT
Klf15 – reverse	CCTGGAACAAAGCTGGACTC
Fkbp5 – forward	GCTGCAAGACCCGTACCCT
Fkbp5 – reverse	TTCCACTCAGGTAACTCTTCCACA
Pck1 – forward	CTATGCTCTCCCTCACGCCA
Pck1 – reverse	TCACGCACGATTTCCCTCTC
Gilz – forward	AGTGAGGACCGGCTACTGTG
Gilz – reverse	GATCAAACGCTTGCGAATCT
G6pd – forward	TGGTGCCCACCTTTCAGTTG
G6pd – reverse	GTCAGGGACTGGCTGTAACC
Cyp3a11 – forward	ACCGAAATGCTCAGTGGGTTACCTA
Cyp3a11 – reverse	GGAACAGAAGGCTTGCGAGTCA
β-actin – forward	GTGCTCCTAGCAATCAGCTT
β-actin – reverse	CAGTGCCTAAAAATGGCAGAGG

References

1. Chondrex. Collagen Antibody Induced Arthritis (CAIA) in Mice, https://www.chondrex.com/documents/Mouse-CAIA.pdf (2020, accessed 16-09-2022).
2. Khachigian LM. Collagen antibody-induced arthritis. *Nat Protoc* 2006; 1: 2512-2516. 2007/04/05. DOI: 10.1038/nprot.2006.393.
3. Brand DD, Latham KA and Rosloniec EF. Collagen-induced arthritis. *Nat Protoc* 2007; 2: 1269-1275. 2007/06/05. DOI: 10.1038/nprot.2007.173.
4. Terato K, Harper DS, Griffiths MM, et al. Collagen-induced arthritis in mice: synergistic effect of E. coli lipopolysaccharide bypasses epitope specificity in the induction of arthritis with monoclonal antibodies to type II collagen. *Autoimmunity* 1995; 22: 137-147. 1995/01/01. DOI: 10.3109/08916939508995311.
5. Timmermans S, Souffriau J and Libert C. A General Introduction to Glucocorticoid Biology. *Front Immunol* 2019; 10: 1545. 2019/07/25. DOI: 10.3389/fimmu.2019.01545.
6. Oray M, Abu Samra K, Ebrahimiadib N, et al. Long-term side effects of glucocorticoids. *Expert Opin Drug Saf* 2016; 15: 457-465. 2016/01/21. DOI: 10.1517/14740338.2016.1140743.
7. Hemshekhar M, Anaparti V, Hitchon C, et al. Buprenorphine Alters Inflammatory and Oxidative Stress Molecular Markers in Arthritis. *Mediators Inflamm* 2017; 2017: 2515408. 2017/06/03. DOI: 10.1155/2017/2515408.
8. Jirkof P, Arras M and Cesarovic N. Tramadol:Paracetamol in drinking water for treatment of post-surgical pain in laboratory mice. *Applied Animal Behaviour Science* 2018; 198: 95-100. DOI: 10.1016/j.applanim.2017.09.021.
9. Evangelista Vaz R, Draganov DI, Rapp C, et al. Preliminary pharmacokinetics of tramadol hydrochloride after administration via different routes in male and female B6 mice. *Vet Anaesth Analg* 2018; 45: 111-122. 20170816. DOI: 10.1016/j.vaa.2016.09.007.
10. Jansen DT, el Bannoudi H, Arens R, et al. Abatacept decreases disease activity in a absence of CD4(+) T cells in a collagen-induced arthritis model. *Arthritis Res Ther* 2015; 17: 220.
11. Bachmanov AA, Reed DR, Beauchamp GK, et al. Food Intake, Water Intake, and Drinking Spout Side Preference of 28 Mouse Strains. *Behavior Genetics* 2002; 32: 435-443.
12. EchoMRI™. EchoMRI™-100H and EchoMRI™-130 Body Composition Analyzers for Live Small Animals (Mice) and Organs, http://www.echomri.com/Body_Composition_Mice_2MHz.aspx (2016, accessed 16-09-2022).
13. CrystalChem. Ultra Sensitive Mouse Insulin ELISA kit, https://www.crystalchem.com/ultra-sensitive-mouse-insulin-elisa-kit.html (accessed 16-09-2022).
14. Immunodiagnosticsystems. Corticosterone HS (High Sensitivity) EIA, https://www.idsplc.com/products/corticosterone-hs-high-sensitivity-eia/ (accessed 16-09-2022).
15. Moore AR, Allden S, Bourne T, et al. Collagen II antibody-induced arthritis in Tg1278TNFko mice: optimization of a novel model to assess treatments targeting human TNFα in rheumatoid arthritis. *Journal of Translational Medicine* 2014; 12.
16. Maleitzke T, Weber J, Hildebrandt A, et al. Standardized protocol and outcome measurements for the collagen antibody-induced arthritis mouse model. *STAR Protoc* 2022; 3: 101718. 20220922. DOI: 10.1016/j.xpro.2022.101718.
17. Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 1980; 284.
18. Salassa RM, Bennett WA, Keating FR, et al. Postoperative acute adrenal cortical insufficiency. *JAMA* 1957; 165: 41-44.
19. Evanson NK, Tasker JG, Hill MN, et al. Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. *Endocrinology* 2010; 151: 4811-4819. 2010/08/13. DOI: 10.1210/en.2010-0285.

20. Lenzen S and Bailey CJ. Thyroid Hormones, Gonadal and Adrenocortical Steroids and the Function of the Islets of Langerhans. *Endocrine Reviews* 1984; 5: 411-434.
21. Schakman O, Gilson H and Thissen JP. Mechanisms of glucocorticoid-induced myopathy. *J Endocrinol* 2008; 197: 1-10. 2008/03/29. DOI: 10.1677/JOE-07-0606.
22. Wong S, Tan K, Carey KT, et al. Glucocorticoids stimulate hepatic and renal catecholamine inactivation by direct rapid induction of the dopamine sulfotransferase Sult1d1. *Endocrinology* 2010; 151: 185-194. 2009/12/08. DOI: 10.1210/en.2009-0590.
23. Bose SK, Hutson I and Harris CA. Hepatic Glucocorticoid Receptor Plays a Greater Role Than Adipose GR in Metabolic Syndrome Despite Renal Compensation. *Endocrinology* 2016; 157: 4943-4960. 2016/11/03. DOI: 10.1210/en.2016-1615.
24. Skauby RH, Bergan S, Andersen AM, et al. In vitro assessments predict that CYP3A4 contributes to a greater extent than CYP3A5 to prednisolone clearance. *Basic Clin Pharmacol Toxicol* 2021; 129: 427-436. 2021/08/17. DOI: 10.1111/bcpt.13645.
25. Myers LK, Kang AH, Postlethwaite AE, et al. The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. *Arthritis & Rheumatism* 2000; 43: 2687-2693.
26. Jirkof P, Durst M, Klopfleisch R, et al. Administration of Tramadol or Buprenorphine via the drinking water for post-operative analgesia in a mouse-osteotomy model. *Sci Rep* 2019; 9: 10749. 20190724. DOI: 10.1038/s41598-019-47186-5.
27. Hawkins P, Armstrong R, Boden T, et al. Applying refinement to the use of mice and rats in rheumatoid arthritis research. *Inflammopharmacology* 2015; 23: 131-150. 20150714. DOI: 10.1007/s10787-015-0241-4.