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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.

Keywords

Analgesia, collagen antibody-induced arthritis, endocrinology, immunology, physiology, prednisolone, techniques

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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 collagen³ resulting in arthritis onset after four 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).

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GCs are very potent anti-inflammatory drugs used to treat a variety of diseases, including allergies, chronic inflammatory diseases and autoimmune diseases such as 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 do 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 (TP) were selected, since no clearly defined anti-inflammatory effects of these compounds have 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 determined whether the combination of TP interferes with the CAIA model when testing the anti-inflammatory effects of the GC drug prednisolone (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 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.

Methods

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, 16 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), four mice per group. Mice receiving only the Veh 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 Center (LUMC). Each cage housed four mice and contained special bedding material (Enrich-n'Pure; The Andersons, Maumee, OH), nesting material (Nesting Cup; Carfil, Oud-Turnhout, Belgium) and a GLP fun tunnel (LBS Biotech, Horsham, UK) as enrichment. The mice received pelleted food ad libitum (RM3 diet, irradiated 9 kGy; SDS Diets, Witham, UK) as well as DietGel recovery cups (ClearH2O, Westbrook, ME) to ensure sufficient hydration and nutrient intake. The light cycle was set to mimic sunrise at 06:30am and sunset at 6:30pm. 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%).

Ethical statement

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

Arthritis induction and scoring

Mice were approximately eight weeks old upon arrival at the animal facility. After a week of acclimatisation, on day 1 of the study, mice were restrained in a tail-first restrainer and received 1.5 mg of CAIA cocktail (Chondrex, Redmond, WA) intravenously via the tail vein. On day 4, the mice were scruff restrained and received 50 µg LPS (Chondrex) through intraperitoneal injection. On day 5, the mice were given DietGel®

Recovery (ClearH₂O) to support their recovery from the LPS injection. Arthritis was scored daily from day 6 until day 15 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 is 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 to treatment allocation. Treatment itself and data analysis were performed in a non-blinded manner.

Treatments and handlings

Pred- and Veh-treated groups were divided over TP-treated cages and non-TP treated cages to minimise cage effects. TP was administered from day 6 onwards via drinking water containing 1 mg/mL tramadol hydrochloride (Merck, Rahway, NJ) and 3.5 mg/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 the tramadol. Based on the average consumption of an individual BALB/cAnNCrl mouse of 6 mL/30 g 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, St Louis, MO) has poorly solubility in water and was therefore reconstituted in 10% EtOH and administered at a concentration of 25 mg/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 day 7 until day 15 via intraperitoneal injection upon scruff restraining. Blood was collected by tail-vein sampling on day 0 (75 µL), day 10 (75 µL) and day 14 (40 µL) by restraining the mice in a tail-first restrainer. The day 14 bleeding was performed after six hours of fasting (water was available), which is required for reliable determination of insulin serum levels. On day 15, all mice were sacrificed by gradual exposure to carbon dioxide, and blood was collected through heart puncture. Blood on day 0 and day 10 was collected in clotting activator tubes (Microvette® CB300; Starstedt, Nümbrecht, Germany) resulting in serum; blood on day 14 was collected in sodium heparin capillaries (Carl Roth, Karlsruhe, Germany) resulting in plasma.

Humane endpoints

Humane endpoints were set for weight loss and arthritis score. Body weight was monitored by weighing the mice daily. 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 three paws or high arthritis in four paws (see 'Arthritis induction and scoring'). Reaching an arthritis score of 45 would severely impair normal behaviour (such as washing, eating, walking and 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.

Body composition analyses

On day 0 and day 15, 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 day 15, after the mice were sacrificed, the thymus and left adrenal gland were collected and weighed.

Liver and muscle gene expression analyses

On day 15, liver and quadriceps muscle tissue were collected in TRIzol (Invitrogen, Carlsbad, CA) and mRNA was isolated using the miRNeasy mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Samples were treated with DNase using DNA-free™ (Ambion, Austin, TX). The cDNA synthesis was performed using iScript (Bio-Rad, Hercules, CA) using 1 µg RNA per sample. For quantitative polymerase chain reaction (qPCR), 10 µM of forward and 10 µM of reverse primer (see Table 1 for sequences), 12.5 µL of iQ SYBR Green Supermix (Bio-Rad) and 1 µL cDNA were added to the qPCR reaction mixture. The qPCR reactions were performed on a MyiQ™ single-colour real-time PCR detection system (Bio-Rad) with denaturation for three minutes at 95°C and 40 cycles of 15 seconds at 95.5°C, 15 seconds at 60°C and 30 seconds 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 normalised using the expression of β -actin. The fold change per sample (compared to the respective control group) was calculated using the $\Delta\Delta$ Ct method. In each experiment, four

Table 1. Quantitative polymerase chain reaction primer sequences.

Gene	Sequence
<i>Redde1</i> – forward	GTCCAAAGCCTCAGAGTCGTTT
<i>Redde1</i> – reverse	AGCCTTTCTCATTGGCACTGTC
<i>Foxo3</i> – forward	AGCATTCAACGCCAGGTTT
<i>Foxo3</i> – reverse	CGAGTCTGTCAGTTCAATACCAA
<i>Gs</i> – forward	CATCACCTGGTACAACCTTT
<i>Gs</i> – reverse	CGGCAACTAAACTCAGAAAC
<i>Klf15</i> – forward	TCTTCATGCCACCATCATGT
<i>Klf15</i> – reverse	CCTGGAACAAAGCTGGACTC
<i>Fkbp5</i> – forward	GCTGCAAGACCCGTACCCT
<i>Fkbp5</i> – reverse	TTCCACTCAGGTAACCTTCCACA
<i>Pck1</i> – forward	CTATGCTCTCCCTCACGCCA
<i>Pck1</i> – reverse	TCACGCACGATTTCCTCTC
<i>Gilz</i> – forward	AGTGAGGACCGGCTACTGTG
<i>Gilz</i> – reverse	GATCAAACGCTTGCGAATCT
<i>G6pd</i> – forward	TGGTGCCACCTTTCAAGTTG
<i>G6pd</i> – reverse	GTCAGGGACTGGCTGTAACC
<i>Cyp3a11</i> – forward	ACCGAAATGCTCAGTGGGTACCTA
<i>Cyp3a11</i> – reverse	GGAACAGAAGGCTTGCGAGTCA
β -actin – forward	GTGCTCCTAGCAATCAGCTT
β -actin – reverse	CAGTGCCTAAAAATGGCAGAGG

mice were used for each treatment group (except for the Veh + TP group, for which three mice were used). All reactions were performed in duplicates. The sequences of all qPCR primers are depicted in Table 1.

Enzyme-linked immunosorbent assays

Plasma collected on d14 was used to determine insulin levels by using the ultra-sensitive mouse insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Downers Grove, IL), following the manufacturer's protocol.¹³ Serum collected on day 15 was used to measure corticosterone levels using the HS enzyme immunoassay kit (Immunodiagnostic Systems, The Boldons, UK), following the manufacturer's protocol.¹⁴

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 the 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 wanted 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 was 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 v9 (GraphPad Software, San Diego, CA). Note should be taken when interpreting the *p*-values, since the Veh + TP-treated group included only three mice and therefore comparisons to this group cannot reach statistical significance.

Results

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 day 4, after which a rapid decline was observed (Figure 1(a) and (b)) and the mice were considerably less active. After 48 hours, the mice should regain 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 stabilise on day 7, 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 have resulted 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 day 6. 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 three mice in the Veh + TP group, whereas all other experimental groups contained four mice.

The mice started showing signs of arthritis on day 6. Before day 6, 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 less active, weight was the only parameter measured

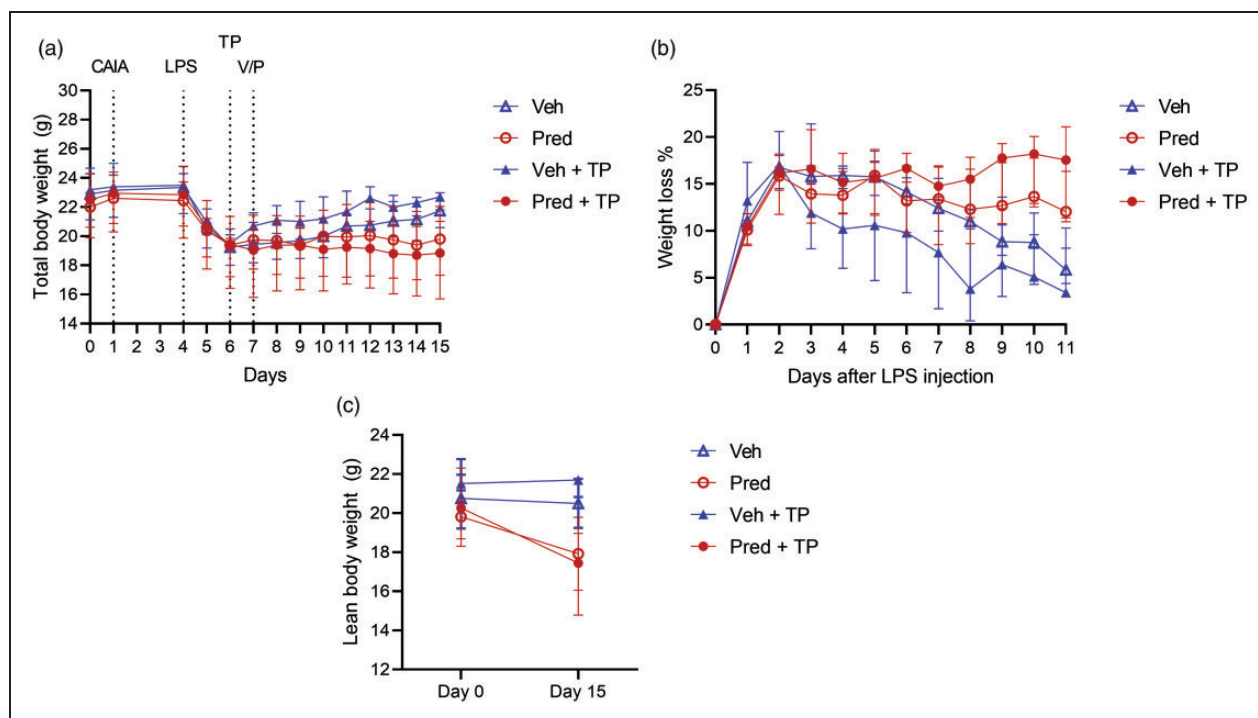


Figure 1. Body weight of the mice over the course of the study. (a) Total body weight in grams of the mice as measured each study day. (b) Weight loss % in comparison to the weight on the day of LPS injection and (c) Lean body weight in grams as calculated by subtracting body fat weight from the total 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.

to minimise 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 intraperitoneal (Veh) injections were observed.

TP alters arthritis development and reduces Pred efficacy

From day 8 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 day 7, reaching scores of approximately 0 at day 11 (Figure 2(a)). Interestingly, in mice receiving TP, the arthritis scores were modest and reached maximal values of approximately 10 at day 8 (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)).

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 apoptosis¹⁷ and adrenal gland atrophy.¹⁸ We therefore analysed 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 (Figure 3(a) and (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 axis.¹⁹ We indeed also observed a Pred-induced reduction in corticosterone levels 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

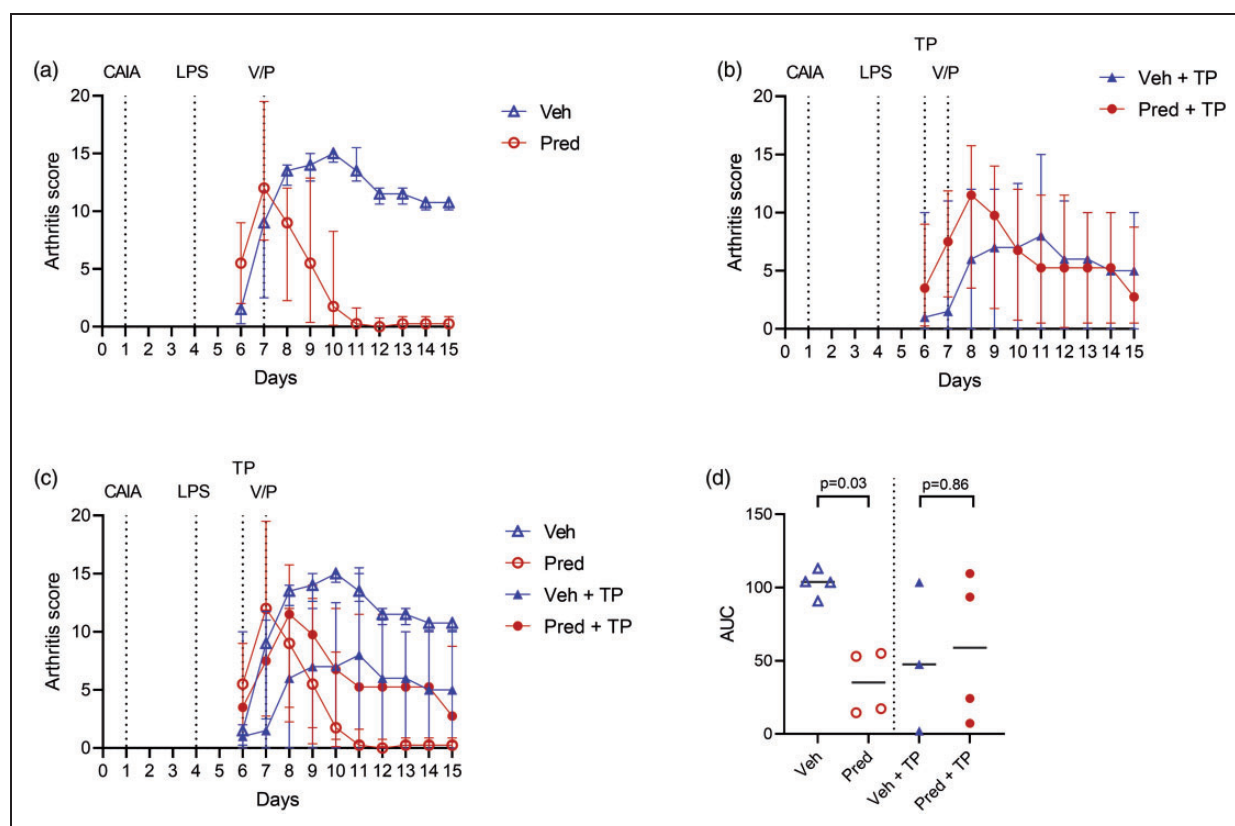


Figure 2. Arthritis scores of mice during the course of the study. (a) Arthritis scores of Veh- and Pred-treated mice. Each line indicates the median values per group; interquartile ranges are depicted. (b) Arthritis scores of Veh + TP- and Pred + TP-treated mice. Each line indicates the median values 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 (a) and (b) for visualisation purposes and (d) Area under the curve (AUC) values of arthritis scores. Individual values and medians are depicted.

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)).

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 analysed 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, such as *Fkbp5* and *Gilz*, as well as genes involved in GC-induced hyperglycaemia, such as *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)).

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

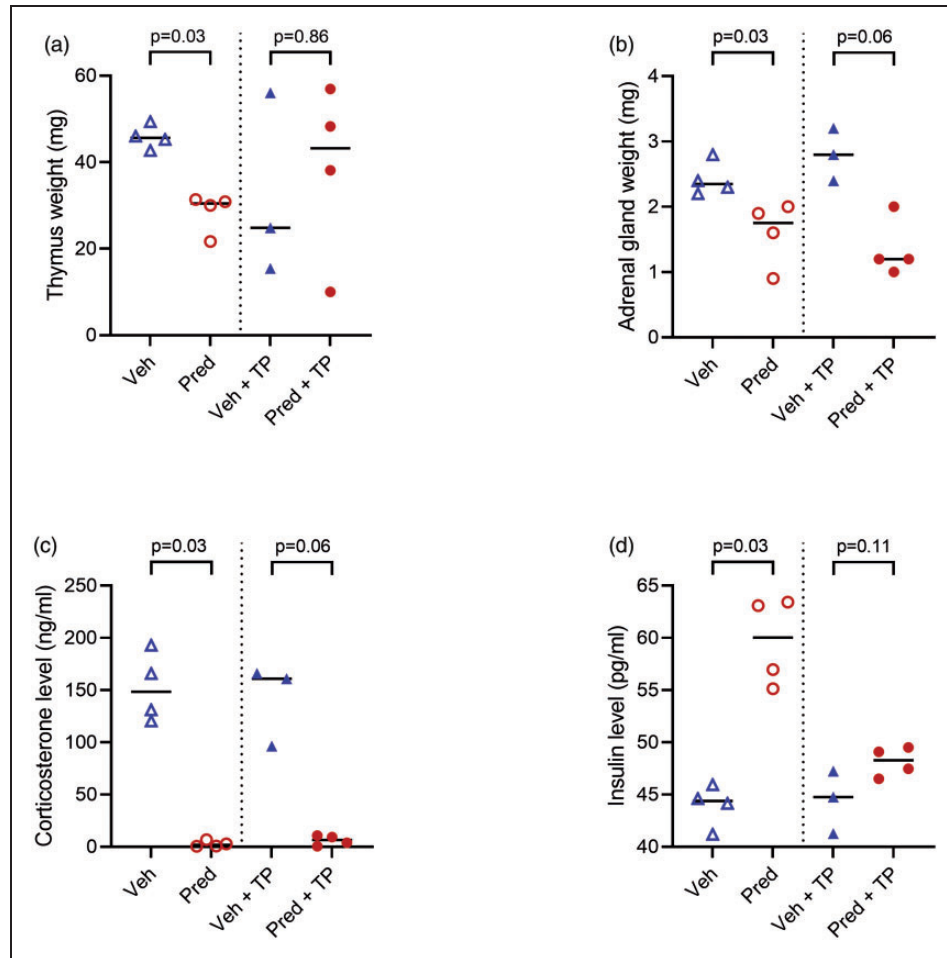


Figure 3. TP affects Pred-induced effects. (a) Thymus weight in milligrams as determined after thymus isolation on day 15. (b) Adrenal gland weight in milligrams as determined after adrenal gland isolation on day 15. (c) Corticosterone levels in serum collected on day 15 and (d) Insulin levels in plasma collected on day 14 after a six-hour fasting period from 7:00am until 1:00pm. Individual values and medians are depicted.

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 clearly 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 only tramadol 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 the LPS injection. Even though tramadol-induced effects on body weight and nutrient intake have not been found to be an aggravated issue during this pilot study, it should be taken into account when considering tramadol as an 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

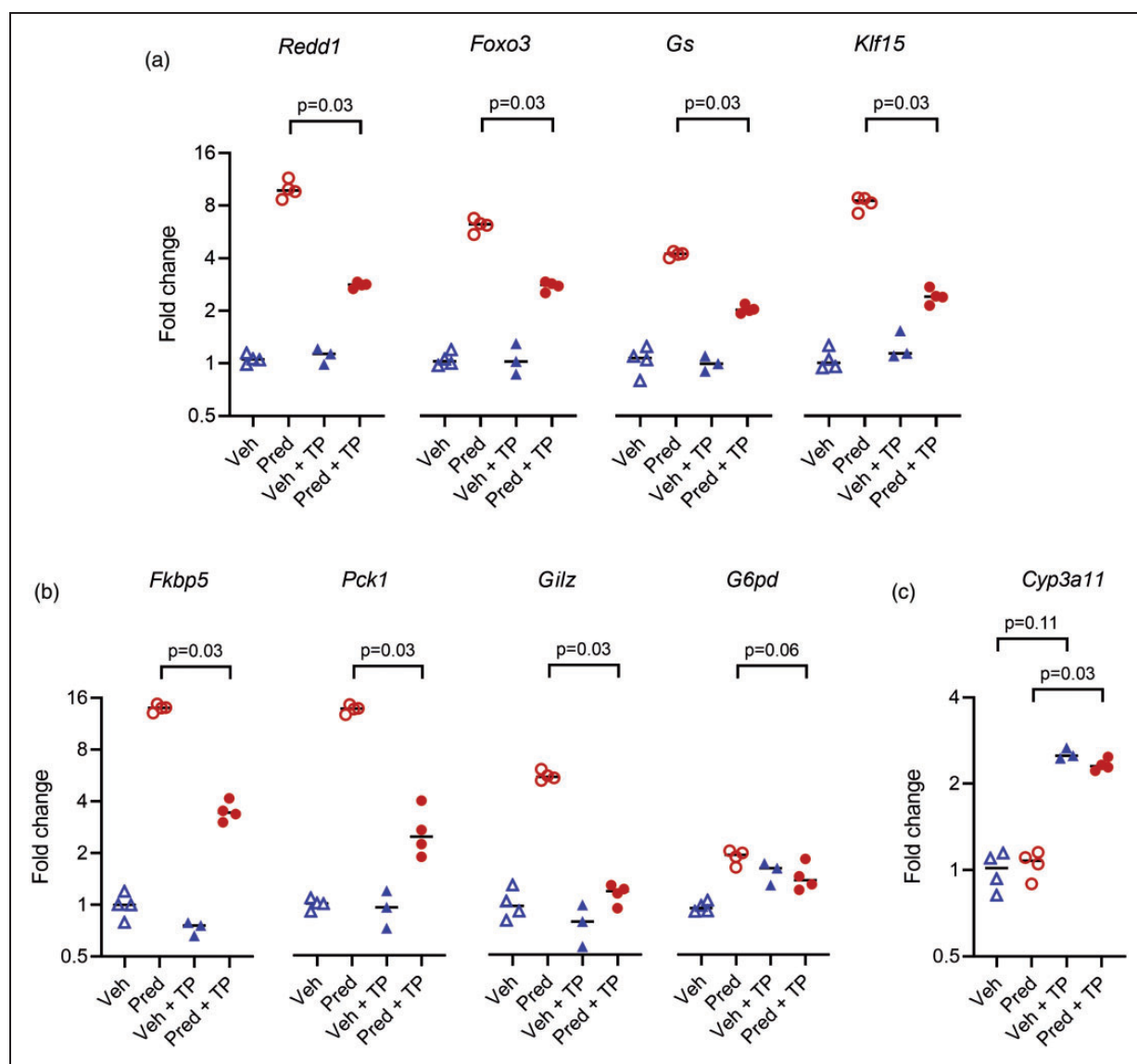


Figure 4. TP effects on the expression of GR-target genes. (a) Expression of GR-target genes in quadriceps muscle tissue and (b) Expression of GR-target genes in liver tissue and (c) Expression of *Cyp3a11* in liver tissue. Individual values and medians are depicted. GR: glucocorticoid receptor.

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 hyperglycaemia. 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 behavioural assessments to determine TP efficacy. So, adequacy of TP on ameliorating murine well-being cannot be confirmed. It is important to consider that only one dose of TP and Pred was analysed 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 doses.

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 into the possible confounding factors influencing research results when refining animal models.

Declaration of conflicting interests

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Le traitement par le tramadol/paracétamol atténue le développement de l'arthrite induite par les anticorps du collagène et interfère avec le traitement par prednisolone chez la souris

Résumé

Le modèle de l'arthrite induite par les anticorps du collagène (CAIA) est très efficace pour induire l'arthrite, ce qui en fait un modèle attrayant pour le criblage de composés thérapeutiques tels que les glucocorticoïdes (GC). L'administration d'analgésiques est souhaitable du fait de l'inconfort dans ce modèle mais ils ne doivent pas interférer avec le modèle ni les produits thérapeutiques testés. Dans la présente étude, nous avons étudié l'effet de 1 mg/ml de tramadol et de 3,5 mg/ml de paracétamol (TP) sur l'arthrite induite par les anticorps du collagène CAIA chez des souris mâles BALB/cAnNCrI et l'interférence possible de l'analgésie au TP avec l'activité du traitement par un GC, la prednisolone (Pred). Nos résultats ont montré que le TP abolissait l'amélioration induite par la Pred de la CAIA, ainsi que plusieurs autres effets induits par la Pred, tels que la réduction du poids du thymus et l'augmentation du niveau d'insuline. Ceci résulte très probablement des effets du TP sur le métabolisme hépatique de ce médicament, car il augmentait fortement l'expression de *Cyp3a11* dans le foie. Dans l'ensemble, nous concluons que l'analgésie au TP n'est pas adaptée au modèle de CAIA chez les souris mâles BALB/cAnNCrI, en particulier lors de l'évaluation des effets des GC tels que la Pred.

Tramadol-/Paracetamol-Behandlung hemmt die Entwicklung von Kollagen-Antikörper-induzierter Arthritis und greift in die Prednisolon-Behandlung bei Mäusen ein

Abstract

Aufgrund seiner hohen Wirksamkeit bei der Auslösung von Arthritis ist das Modell der Kollagen-Antikörper-induzierten Arthritis (CAIA) attraktiv für das Screening therapeutischer Wirkstoffe wie Glukokortikoide (GC). Die Stärke der Beschwerden in diesem Modell macht die Verabreichung von Analgetika wünschenswert, vorausgesetzt jedoch, dass diese nicht mit dem Modell oder den getesteten Therapeutika interferieren. In der vorliegenden Studie untersuchten wir die Wirkung von 1 mg/ml Tramadol und 3,5 mg/ml Paracetamol (TP) auf CAIA bei männlichen BALB/cAnNCrI-Mäusen und den möglichen Einfluss der TP-Analgesie auf die Wirkung des GC-Medikaments Prednisolon (Pred). Unsere Ergebnisse zeigten, dass TP die Pred-induzierte Verbesserung der CAIA ebenso wie mehrere andere Pred-induzierte Effekte, wie die Verringerung des Thymusgewichts und den Anstieg des Insulinspiegels, aufhob. Dies ist höchstwahrscheinlich auf die Auswirkungen von TP auf den hepatischen Metabolismus dieses Medikaments zurückzuführen, da es die *Cyp3a11*-Expression in der Leber stark erhöht. Insgesamt kommen wir zu dem Schluss, dass die TP-Analgesie für das CAIA-Modell bei männlichen BALB/cAnNCrI-Mäusen nicht geeignet ist, insbesondere wenn es um die Bewertung der Auswirkungen von GC wie Pred geht.

El tratamiento con tramadol/paracetamol atenúa el desarrollo de artritis inducida por anticuerpos de colágeno e interfiere con el tratamiento con prednisolona en roedores

Resumen

El modelo de artritis inducida por anticuerpos de colágeno (CAIA) es muy eficaz para inducir artritis, lo que lo convierte en un modelo atractivo para el cribado de compuestos terapéuticos como los glucocorticoides (GC). La gravedad de las molestias en este modelo hace deseable la administración de analgésicos, pero es un requisito previo que estos no interfieran con el modelo ni con los compuestos terapéuticos probados. En el presente ensayo, estudiamos el efecto de 1 mg/mL de tramadol y 3,5 mg/mL de paracetamol (TP) sobre el CAIA en ratones machos BALB/cAnNCrl y la posible interferencia de la analgesia de TP con la actividad del fármaco GC prednisolona (Pred). Nuestros resultados mostraron que la TP abolía la mejoría del CAIA inducida por la Pred, así como otros efectos inducidos por la Pred, como la reducción del peso del timo y el aumento del nivel de insulina. Lo más probable es que esto se deba a los efectos de la TP sobre el metabolismo hepático de este fármaco, ya que aumentó significativamente la expresión de *Cyp3a11* en el hígado. En conjunto, concluimos que la analgesia con TP no es adecuada para el modelo CAIA en ratones machos BALB/cAnNCrl, especialmente cuando se evalúan los efectos de GC como Pred.