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Research article

Effects of dexamethasone and acetylsalicylic acid on inflammation caused by Complete Freund's adjuvant in the naked mole rat (*Heterocephalus glaber*)

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ABSTRACT

The naked mole rat (NMR) is a fossorial rodent that has been observed to have a unique nociceptive system in comparison to others. In this study, we explored on characterization of chronic inflammation in the NMR using Complete Freund's adjuvant (CFA) and investigated the effects of dexamethasone and acetylsalicylic acid on the resulting inflammation. The NMRs were injected with 0.1 ml of CFA subcutaneously in the right hind paw, and an equivalent volume of normal saline was injected to the control group. Swelling of the injected right hind limb was observed within 24 h of injection, which involved the tibiotarsal joint, palmar surface and the digits of the injected paw. Swelling persisted for 6 weeks of experimentation and peaked between day 14 and 21. The resulting inflammation affected the mobility, stance and joint rigidity of CFA treated NMRs in comparison to the control group. Treatment of the chronic phase of the inflammation from the 11th day with dexamethasone and acetylsalicylic acid showed no statistical significance in paw circumference compared to the control group, other than on a few, negligible occasions. The present data showed that CFA was able to induce chronic inflammation in the NMR, and the NMR could thus be established as a model for chronic inflammation. There is, however, need for more sensitive parameters to evaluate the effects of anti-inflammatory drugs.

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disorder of unknown etiology, which is characterized by chronic inflammation and infiltration of autoimmune cells (Choudhary et al., 2018). Chronic inflammation affects multiple joints and causes cartilage erosion that result in a lifelong progressive disease, which produces significant morbidity and premature mortality. In a severe state, RA may lead to debilitating morbidity, major economic costs due to both health care expenditures and lost productivity (Fischer et al., 2017), and may also lead to the development of cancer (Coussens and Werb, 2002), cardiovascular diseases (Libby, 2012), and neurodegenerative diseases (Tak et al., 2001).

There is no cure for chronic inflammation in RA (Walsh and McWilliams, 2014). Current therapy includes nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease modifying anti-rheumatic drugs (DMARDs), or anti-cytokine drugs (Majithia and Geraci, 2007), which aim to reduce pain, fatigue, and disability by mainly focusing on controlling synovitis. Glucocorticoids contribute an important part of the neuroendocrine response by influencing inflammation. They are also involved in the regulation of different physiological functions during hyperalgesia (Yoshida et al., 2005). Dexamethasone injections in CFA has been observed to reduce all arthritic symptoms including histological changes during the period of drug administration (Francischi et al., 1997; Lam and Ng, 2010), but other reports have concluded that their effects are time related (Church and Miller, 1978; Hirschelmann and Bekemeier, 1986; Houshyar et al., 2000).

Complete Freund's adjuvant (CFA) induced arthritis or inflammation has been used to study chronic pain in rats, mice and guinea pigs as animal models of rheumatoid arthritis (Choudhary et al., 2018). It involves injection of CFA systemically or intraarticularly, where the resulting non-infectious arthritis macroscopically manifests as swelling of articulated joints and affected gait and mobility. It is characterized by a rapid onset with either local inflammation or progression to poly-articular inflammation depending on where the adjuvant is delivered (Hawkins et al., 2015). The symptoms of arthritis can be observed after about 24 h, and normally peak around 10–14 days post-injection.

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The induced arthritis may become severe leading to permanent joint malformations, including ankyloses. Other inflammatory reactions like lymphocyte infiltration and cartilage degradation are also present (Choudhary et al., 2018).

Of the mentioned species, specific strains and stocks of mice and rats have been well established as models for RA, but none of them has completely reflected all the features of RA in humans. This raises the need to study chronic pain and inflammation in other rodent species for comparison purposes. Besides, not all rodents are equally vulnerable to experimental arthritis causing agents (Tuncel et al., 2016). There is also a lack of efficiency in the existing drugs used in management of chronic inflammation and their use is far from satisfactory, since RA manifests with different signs in patients. In order to develop better management practices for the condition, there is need for more studies that may require the use of animals to demystify RA in humans.

The naked mole-rat (NMR) (*Heterocephalus glaber*) is a sub-terranean rodent belonging to the family Bathyergidae, endemic in the arid regions of East Africa. It is a rather unique mammal compared to other mammals and has over time attracted the attention of many scientists who want to study it with the intention of making it a model for biomedical research (Smith et al., 2015). NMRs have shown resistance to experimentally induced tumors and can survive under low oxygen levels due to their hemoglobin's high affinity for oxygen. They have few c-fibers, which lack the neurotransmitter substance P (SP) in the skin. SP is involved in sending pain signals to the central nervous system, and lack of SP makes the NMR behaviorally insensitive to itch induced by capsaicin, ammonia fumes and histamine. They have also shown insensitivity to pain induced by acid and acidic fumes (Grimes et al., 2014; Kim et al., 2011; Smith et al., 2015). The naked mole rat was chosen for this study because its nociceptive system appears to be unique, according to previous findings (Kanui et al., 1993; Kim et al., 2011; Park et al., 2008; Towett and Kanui, 1993; Towett et al., 2006, 2009), in contrast to those of other mammals used for pain research. To our sincere knowledge, no data is available on how the naked mole rats react to injection of CFA or what effects drugs such as dexamethasone and acetylsalicylic acid might have on the plausibly induced pain and inflammation. The aim of the present study was to establish the naked mole rat as a model of CFA induced arthritis and to evaluate the anti-inflammatory effects of the glucocorticoid dexamethasone and the NSAID acetylsalicylic acid on chronic inflammation in the NMR. It was hypothesized that injection of CFA in the right hind paw would increase paw circumference and arthritic score compared to animals not injected with CFA. It was also hypothesized that dexamethasone and acetylsalicylic acid would reduce paw circumference and arthritic scores in comparison to CFA injected animals not treated with the drugs.

2. Materials and methods

2.1. Animals

NMRs were captured in Kibwezi, Makueni County, Kenya. The experimental procedures were performed after ethical approval of the Institutional Animal Use and Care Committee at the Faculty of Veterinary Medicine, University of Nairobi. The experimental procedures were conducted in accordance with the prevention of cruelty to animals act, chapter 360 laws of Kenya and European Union Directive (2010)/63/EU wherever applicable.

The animals were under health surveillance by a veterinarian, and found to be in good health throughout the entire duration of the experimental period. A total of 54 male and female adult animals varying in mass between 25-35 g were captured and used in the study. Each animal was used only once, and euthanized after testing was completed.

2.2. Housing and husbandry

Animals captured from one colony were housed together in designated cages. The cages were made of acrylic glass (plexiglass) covered with an opaque paper cover to keep the cages dark as required. Each cage comprised of two compartments, with an interconnecting tunnel that measured 20 × 10 × 10 cm to mimic burrows. Coarse sawdust and wood shavings mixed with sand were used as bedding and changed once a week to ensure the cages were damp-free. Room temperature was maintained at between 28-31°with a 24/0 dark/light cycle. Humidity in the room was maintained at 45–50%, to mimic their subterranean environment and avoid drying and scaling of the skin of the animals. The animals were fed on fresh carrots and sweet potatoes *ad libitum*. No water was provided, as they have not been observed to drink, neither in studies in the wild nor during experimental conditions. The water content in the feed is considered sufficient for hydration. The NMRs were acclimatized to the laboratory conditions for a period of one month before inception of the studies. Over this period and during experimentation the NMRs were handled and weighed daily.

2.3. Study design

After acclimatization the animals were randomly re-distributed into groups of six animals per group (n = 6) and each group was randomly assigned to different treatments. Male and female animals were equally distributed in the groups to avoid any sex related bias. The sample size was based on previous studies (Dulu et al., 2014; Mwobobia et al., 2020). Each group of six was put under either of the following treatment groups; controls (injected with saline), CFA treatment alone, CFA + treatment with saline, CFA treated with dexamethasone (0.3, 1 and 3 mg/kg) and acetylsalicylic acid (25, 50 and 150 mg/kg).

2.4. Procedures and study outline

The animals were injected with 0.1 ml of CFA mixed with phosphate buffer saline in the ratio 1:1 subcutaneously into the right hind paw, while the equivalent volume of normal saline was injected subcutaneously to the control group (Taranov et al., 2016). The NMRs were anaesthetized before injection of CFA and during paw measurements using isoflurane. To investigate the effect of dexamethasone (0.3, 1 and 3 mg/kg) and acetylsalicylic acid (25, 50 and 150 mg/kg), the drugs were administered daily, intraperitoneally from day 11 when there was a peak in paw inflammation (Figure 1).

Paw circumference was measured using a digital Vernier caliper (Silverlinec - 380244) to the nearest 0.01 mm. The circumference of the paw was determined by the formula.

$2*\pi \sqrt{(0.5*(a^2+b^2))}$. Where "a" is radius of dorso-plantar axis and "b" is radius of medio-lateral axis (Tag et al., 2016). Measurements were made twice every week for six weeks.

The extent of paw swelling and edema was scored by the modified method (Di Paola and Cuzzocrea, 2008), with a score ranging from 0 to 3 (0, no swelling; 1, slight swelling and/or erythema; 2, pronounced edematous swelling and 3, severe arthritis and joint rigidity). The injected limb was graded, thus allowing a maximum score of 3. The scoring was done twice per week. Severity of the inflammation was evaluated by two independent observers who were blinded to the treatment groups. The Butler scoring system (Butler et al., 1992) was used for evaluation of mobility, stance and stiffness scores (Table 1). Each animal was observed in a clear acrylic glass box measuring 50cm*38cm*20 cm for 5 min and the observed behaviours were scored.

2.5. Drugs and chemicals

Complete Freund's adjuvant, acetylsalicylic acid and dexamethasone were obtained from Sigma Aldrich, Denmark. Acetylsalicylic acid and dexamethasone were administered intraperitoneally, and the CFA was administered on the plantar subcutaneous surface of the right hind limb. Acetylsalicylic acid was suspended in 0.9% (w/v) saline and fresh preparations were constituted prior to every use.

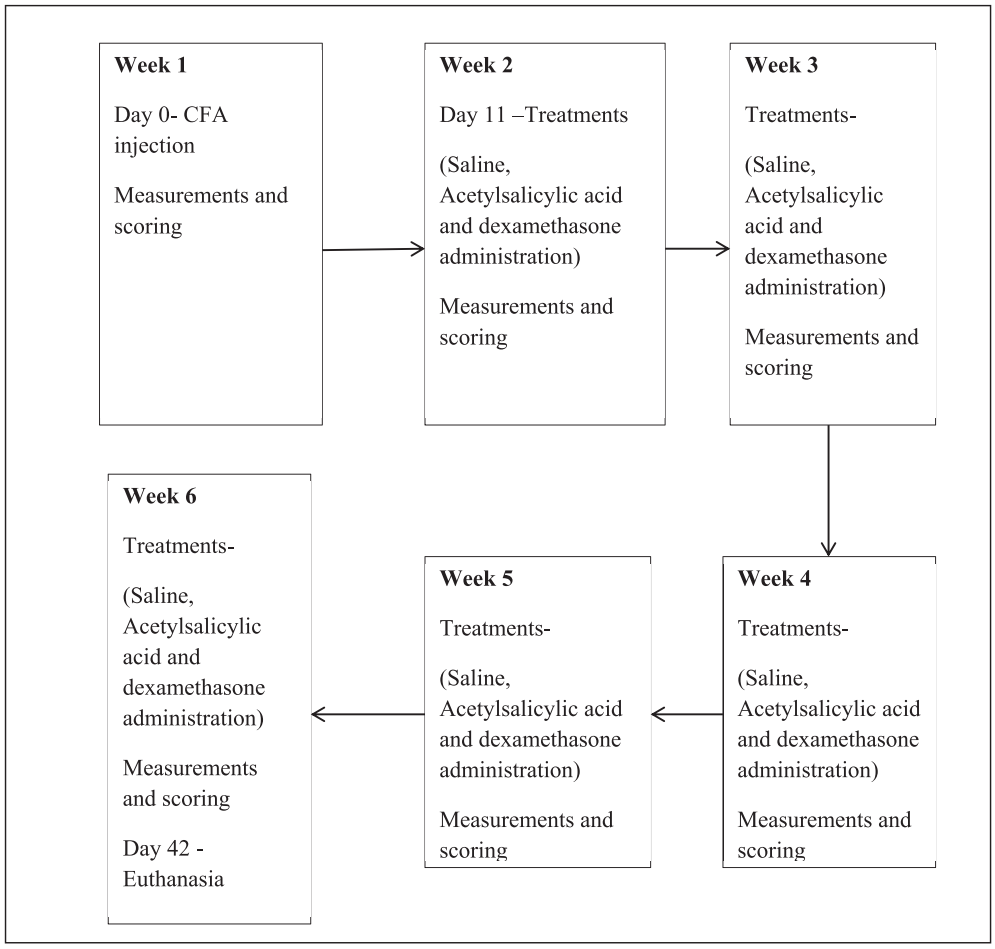

Figure 1. Summary of study outline.

2.6. Data analysis

The data were analyzed using two-way repeated measures ANOVA with Bonferroni's post hoc test in GraphPad Prism version 5.0. Kruskal-Wallis test was used to analyze the paw swelling, edema, mobility, stance and joint stiffness. Data are represented as mean (±SEM). P values < 0.05 were considered statistically significant.

3. Results

3.1. Assessment of Complete Freund's adjuvant-induced arthritis

It was observed that the circumference of the right hind paw injected with CFA increased in size compared to that of controls (Figures 2 and 3). There was a significant difference ($P < 0.0001$) in paw circumference in the CFA treated group over the period of the experiment in comparison to the saline treated group (Figure 3). The swelling of the injected right hind limb was observed within 24 h of injection with 0.1 μl of CFA. Only the right injected paw was observed to increase in size.

The swelling/inflammation of the injected limb involved the tibio-tarsal joint, palmar surface and the digits of the injected paw. The swelling persisted over the period of the experiment (6 weeks) and peaked from day 14 to day 21 (Figure). There was no significant difference ($P > 0.005$) in paw circumference on the contralateral (non-injected) paws in any of the groups.

The paw swelling and edema scoring is shown in Table 2. The control group showed a mild increase in swelling up to Day 7 but no swelling for the rest of the observation period. The CFA treated group was observed to have pronounced edematous swelling from day 0 to Day 7 that progressed to severe rigidity over the remaining period. No other limbs besides the injected ones were observed to be inflamed.

3.2. Effect of dexamethasone and acetylsalicylic acid on paw circumference and swelling

There was no significant difference ($P > 0.05$) in reduction of paw circumference between the CFA control group and the groups treated with dexamethasone in doses of 0.3 and 1 mg/kg. Dexamethasone 3 mg/

Table 1. The score matrix for the Butler et al., 1990 scoring system.

Numerical mobility score	Score
The naked mole rat lies down only	0
The naked mole rat crawls only	1
The naked mole rat walk with difficulty	2
The naked mole rat walk\ and runs with difficulty	3
The naked mole rat walks and runs normally	4
Numerical stance score	
the naked mole rat stands on three paws only	0
the naked mole rat stands with the arthritic paw touching the floor, toes curled under	1
the naked mole rat stands bearing some weight on the arthritic limb	2
The naked mole rat stands bearing some weight equally on all four limbs	3
Numerical joint stiffness score	
Restriction on full range of flexion	1
Restriction of full range of extension	1

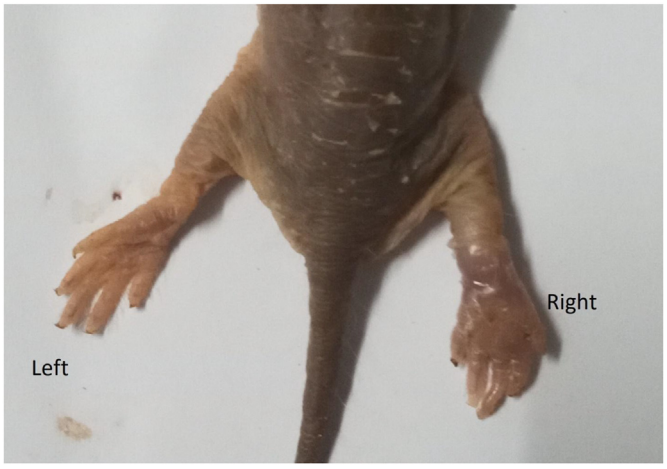

Figure 2. Inflamed right hind leg after injection with CFA compared to left hind foot.

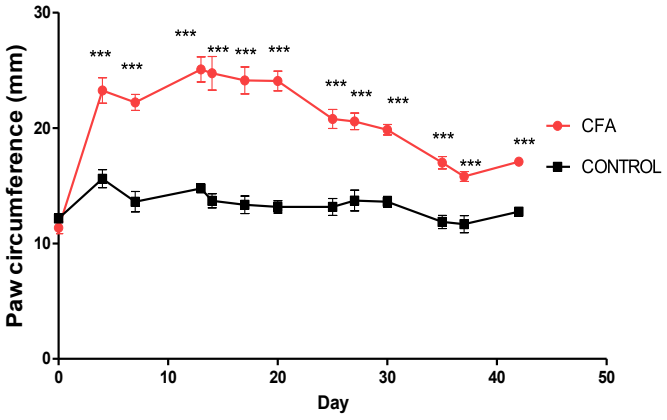

Figure 3. Paw circumference after injection with Complete Freund's adjuvant and saline. Data is represented as means ± SEM.*** Indicates P < 0.0001.

kg, on the other hand, caused a significant difference (P < 0.001) in paw circumference compared to the control group on day 21 (Figure 4).

The macroscopic paw score increased from a score of 2 in the first week to 3 for the remaining period of the experiment in all the three groups (Table 2).

There was no significant difference (P > 0.05) between the group treated with 25 mg/kg acetylsalicylic acid treated group (25 mg/kg) and the CFA control group, but significant difference (P < 0.01) between the control and the groups treated with 50 and 150 mg/kg on day 14 (Figure 5).

The macroscopic paw score increased from a score of 2 after the first week to 3 for the remaining period of the experiment in all the three

Table 2. The median macroscopic score on paw swelling and edema.

	0d	7d	14d	21d	28d	35d	42d
Control	0	1	0	0	0	0	0
CFA	0	2	3	3	2	3	3
CFA + Dexa 0.3	0	2	3	3	2	3	3
CFA + Dexa 1	0	2	3	3	3	3	3
CFA + Dexa 3	0	2	3	3	3	3	3
CFA + ASA 25	0	2	3	3	2	3	3
CFA + ASA50	0	2	3	3	3	3	3
CFA + ASA150	0	2	3	3	3	3	3

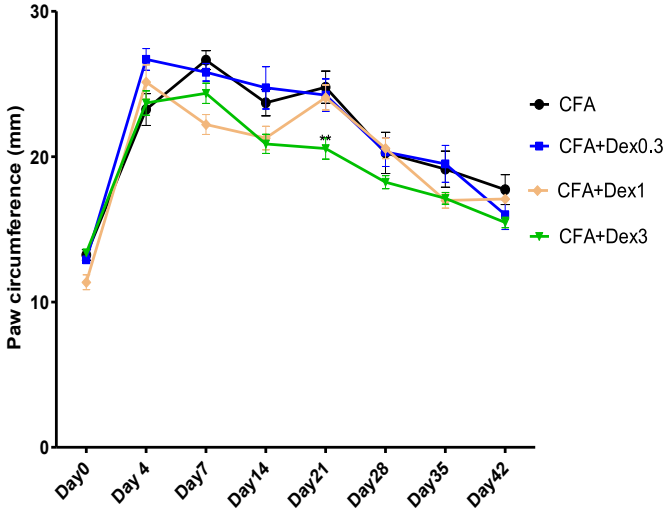

Figure 4. Paw circumference following injection of dexamethasone (0.3, 1 and 3mg\kg body weight) from day 11. Data is represented as means ± SEM. **P < 0.01.

groups, except for the group treated with 25 mg/kg acetylsalicylic acid on Day 28, where the score was 2.

3.3. Body weight

There was no significant change in weight (P > 0.05) over time nor between the groups (Figure 6).

3.4. Mobility, stance and joint stiffness

The effect of CFA injection on mobility, stance and joint stiffness is shown in Table 3. Mobility was at baseline levels for the saline treated control group throughout the study. The mole rats would walk and run normally throughout the period of study with exception of the first week after injection when the injected paw was slightly swollen. In the CFA treated group, the naked mole rats walked and ran with some difficulty throughout the period of study. This similar observation was recorded in CFA induced - dexamethasone and CFA induced - acetylsalicylic acid treated groups.

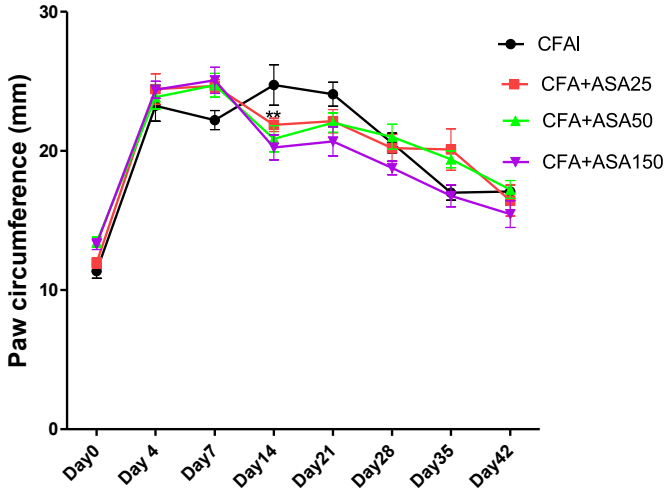

Figure 5. Average Paw circumference ±SEM of the CFA treated group and acetylsalicylic acid 25 mg/kg, 50 mg/kg and 150 mg/kg from day 11. Data is represented as means ± SEM. **P < 0.01.

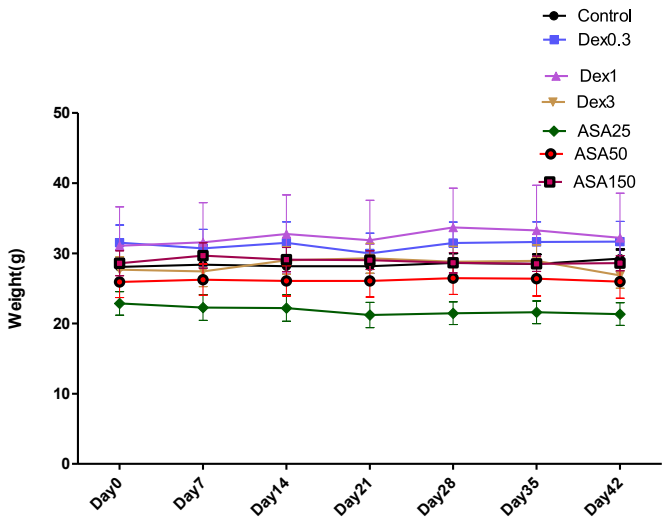

Figure 6. Weight (grams) of naked mole rats over the period of experimentation. Data is represented as mean ± SEM.

The animals in the saline treated group were observed to bear some weight on the injected paw in the first week but for the rest of the period they bore weight equally on all the four limbs. In the CFA treated (including dexamethasone and acetylsalicylic acid treated) groups, the naked mole rats stood with three limbs and would occasionally stand with the arthritic paw touching the ground with the toes curled under. At no point during the study were they observed to bear weight equally on all four limbs. This observation indicated that arthritic scores and the paw macroscopic score increased with time since inoculation with CFA.

No joint stiffness was observed the saline treated control group at any point in the study. Joint stiffness was observed in all the CFA treated groups including those treated with dexamethasone and acetylsalicylic acid on the injected limb only. Other limbs in the CFA treated group were not observed to be stiff at any point in the study.

Table 3. Numerical mobility, stance and joint stiffness scores for the different treatment groups using the Butler scoring system.

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Numerical Mobility score							
Control	4	4	4	4	4	4	4
CFA	4	3	2	3	3	3	3
CFA + Dexa 0.3	4	3	3	3	3	3	3
CFA + Dexa 1	4	3	3	3	3	3	3
CFA + Dexa 3	4	3	3	3	3	3	3
CFA + ASA 25	4	3	3	3	3	3	3
CFA + ASA50	4	3	3	3	3	3	3
CFA + ASA150	4	3	3	3	3	3	3
Stance score							
Control	3	3	3	3	3	3	3
CFA	3	2	1	1	1	1	1
CFA + Dexa 0.3	3	2	1	1	1	1	1
CFA + Dexa 1	3	2	1	1	1	1	1
CFA + Dexa 3	3	2	1	1	1	1	1
CFA + ASA 25	3	2	1	1	1	1	1
CFA + ASA50	3	2	1	1	1	1	1
CFA + ASA150	3	2	1	1	1	1	1
Stiffness score							
Control	0	0	0	0	0	0	0
CFA	0	2	2	2	2	2	2
CFA + Dexa 0.3	0	2	2	2	2	2	2
CFA + Dexa 1	0	2	2	2	2	2	2
CFA + Dexa 3	0	2	2	2	2	2	2
CFA + ASA 25	0	2	2	2	2	2	2
CFA + ASA50	0	2	2	2	2	2	2
CFA + ASA150	0	2	2	2	2	2	2

4. Discussion

This is the first study to report chronic pain and inflammation in the NMR using Complete Freund's Adjuvant.

The NMRs developed inflammation that was characterized by increase in paw circumference and paw swelling within a short time following intraplantar injection of CFA in the right hind limb. The injected limb was also observed to increase in circumference in animals injected with saline, but this did not persist beyond seven days and it was neglectable compared to the swelling observed after CFA injection. There was an initial phase of inflammation in the CFA treated group that peaked in the second week and persisted through to the third week of the experiment. This corresponded to the acute and chronic phase of inflammation (Tuncel et al., 2016). During this acute phase there was progressive inflammation that was not accompanied by a significant decrease in body weight, unlike what is observed in rats and mice (Bolon et al., 2011). Decrease in body weight in rodent models treated with glucocorticoids has been suggested to occur due to dexamethasone interference with their immunity (Zaringhalam et al., 2008). It could be speculated that the lack of body weight loss in the NMR could be caused by a unique mechanism in their metabolism when under physiological stress, as a mechanism to cope with their subterranean environment. It is also possible that the inflammation does not cause enough stress to cause a decrease in body weight.

The increase in paw and joint circumference following administration of an arthritogenic agent has been described as an arthritis disease (Hussein et al., 2012; Kumar et al., 2016). The inflammation developed fully by the 11th day and persisted to the end of the experiment in week six in all the inoculated animals. Our study did not seek to find out the etiology and pathogenesis of the inflammation but we can consider it similar to that observed in other rodents. In rats and mice, the clinical or acute phase is thought to be caused by activation of immune cells, which cause a persistent overproduction of pro-inflammatory (Feige et al., 2000; Rioja et al., 2004) and pro-erosive cytokines (Stolina et al., 2009), as well as abnormal recognition of self-antigens as non-self, due to their similarity with a foreign protein (Bolon et al., 2011). The chronic inflammation which persisted after the initial acute phase in the present study has been observed in other species (Bernardi et al., 2009; Rayhana et al., 2014) and is thought to be caused by a delayed immunological flare in the RA disease (Kuroki et al., 2011; Wang et al., 2012).

Adjuvant induced arthritis models have been used for some time in evaluating and screening of anti-arthritic drugs. Given the diversity of molecular mechanisms and the sensitivity of individuals to different therapeutic agents, the use of different laboratory models is advisable. After successful establishment of CFA in the NMR, we evaluated the effects of dexamethasone and acetylsalicylic acid on their anti-inflammatory effects on the chronic phase of CFA induced inflammation.

Dexamethasone is one of the most common and potent corticosteroids used to reduce inflammation and pain (Zaringhalam et al., 2008). In the present study, dexamethasone at 3 mg/kg was observed to have significant anti-inflammatory effect in the NMR on day 21, which was manifested by a reduction in paw and joint circumference. However, the effect was limited to one single day, and dexamethasone could thus not establish a reduction in circumference of longer duration. The observed effect of dexamethasone could this be occasional and not necessarily reflect the true effect of the drug. In addition, there was no concomitant decrease in the macroscopic paw swelling and arthritic scores.

Higher doses of anti-inflammatory agents have been reported to produce greater reductions in arthritic parameters compared to lower doses (Yoshida et al., 2005). It is therefore possible that even higher doses than those used in the present study would have given better anti-inflammatory effects. The animals were continuously observed for any side effects following drug administration and none were noticed with the 3 mg/kg dose group, but since the doses used were already in the higher range, it was decided not to increase the doses any further to avoid any unwanted side effects from excessively high doses.

Dexamethasone has been reported to regulate pain threshold by attenuating opioid receptor coordination (Yoshida et al., 2005). Previous studies have demonstrated a complex organization in the modulation of antinociception via the opioid receptor system in the NMR (Towett et al., 2006; Dulu et al., 2014). Thus, the lack of effect on dexamethasone could possibly be explained by different mechanism mediated via the opioid system, although more studies are needed to support this hypothesis. Another explanation could simply be that the experimental parameters tested in the present study are not sensitive enough to detect anti-inflammatory effect of dexamethasone on anti-inflammation. This calls for the need for further investigations to identify clinical, physiological and behavioural parameters that could be useful for detecting anti-inflammatory effects. In addition, possible histopathological effects from the CFA-injection as well as from the treatments with dexamethasone and acetylsalicylic acid would likely provide additional information,. This is something we intend to investigate in future studies.

Acetylsalicylic acid was observed not to have any significant effects in the reduction of paw circumference or arthritic scores in the NMR with the lower dose (25 mg/kg), but higher doses (50 mg/kg and 150 mg/kg) were observed to reduce the paw circumference on day 14. Similar to dexamethasone, this subtle and transient effect of acetylsalicylic acid could be occasional and not necessarily reflect the true effect of the drug. Acetylsalicylic acid is one of the oldest NSAIDs and has been used for the longest period in management of chronic inflammation. It has been the longest used NSAID and commonly used as a reference drug for comparing the efficacy of newer drugs and chemicals in the treatment of adjuvant induced arthritis (Paval et al., 2009). It was chosen for this experiment because of its good safety profile in management of RA (Fries and Bruce, 2003; Moore et al., 2015), its low cost and ease of availability.

The lack of effect from acetylsalicylic acid may be due to several reasons. The preferred route of aspirin administration is oral. In our study, intraperitoneal administration was preferred because the NMRs have not been observed to drink any water in experimental setups. Differences in formulations have been reported to have effect on therapeutic success (Fries and Bruce, 2003). Further, the frequency of drug administration was reduced because of the intraperitoneal route of administration, and given the short half-life of acetylsalicylic acid (Fu et al., 1991), this could explain the lack of prolonged effects. Measurement and evaluation of hepatic parameters could have given a better indication of anti-arthritic drugs (Kumar et al., 2016). Early start of treatment during the acute phase could have had some positive effect, given that pro-inflammatory mediators and cytokines including prostaglandins are produced during this time, in contrast to our experiment where we focused on management of the chronic phase of the inflammation. It should also be pointed out that aggressive CFA-induced inflammation has been reported to be unresponsive to relatively weak anti-inflammatory compounds (Bolon et al., 2011). In addition, the lack of effect could also be as suggested in the case with dexamethasone above, that the experimental parameters studied are insufficiently sensitive.

Neither the arthritic scores, mobility, stance, nor joint rigidity (Butler et al., 1992) were observed to be reduced by the anti-inflammatory treatment. Possible reason for the lack of effect has been discussed above, but it should be underlined that the scores applied were discrete with few possible read outs, which makes this type of assessment difficult to use as it requires either a very clear effect or a larger sample size. It may also be that the Butler score is not suitable for assessing inflammation and arthritis in the NMR and that other read-outs should be considered in this species. For future studies, other parameters such as gait analysis and histopathology should be investigated.

It is possible that disparate evolutionary pressures in the NMR to living in underground habitats could result in some of the observations made in our study. It is therefore conceivable that selective pressures have driven evolution of inflammation in this species. With poor gas and heat exchange in their habitats, they are extremely tolerant of hypoxia and hypercapnia (Buffenstein, 1996). They have low basal metabolic rates and resting body temperature (Buffenstein and Yahav, 1991). NMRs have been reported to have higher amounts and larger molecular weight of hyaluronan (HA) in comparison to guinea pigs and mouse. HA is an extracellular matrix which produces different physiological and pathological effects in tissues. This difference has been thought to have an enormous impact on phenomena such as inflammation and aging (Del Marmol et al., 2021). There is also considerable evidence from a study showing that NMRs cells show better cytoprotection and stress resistance than mouse cells (Lewis et al., 2012). Altogether, these adaptive mechanisms indicate that at a cellular and organismal level NMRs are stress resistant to toxins and have enhanced protection against inflammation and neurodegeneration.

In conclusion, this study was able to induce chronic inflammation in the NMR, but there is need for more sensitive parameters to see the effects of anti-inflammatory drugs.

Declarations

Author contribution statement

Jesse Kevin Ndungu Thuo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Philemon Kipkemoi Towett, Titus Ikusya Kanui: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data, Wrote the paper.

Klas S.P. Abelson: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data, Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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