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Refinement of the Collagen Induced Arthritis Model in Rats by Infrared Thermography

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Research Article

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ABSTRACT

Aims: Collagen induced arthritis in rats is an important model for human rheumatoid arthritis. This study was designed to improve and refine this model by use of infrared thermography by measuring surface temperature of hind feet. Our hypothesis is that the local temperature on the feet correlates with other clinical parameters such as clinical score and edema and may serve as a method for quantification of the degree of inflammation.
Study design: Experimental animal study.
Place and Duration of Study: Institute of Biomedicine, University of Aarhus, Denmark between February and March 2010.
Methodology: Arthritis was induced with collagen immunization in sixteen Lewis rats. Four of the animals were treated with dexamethasone to function as negative controls. Clinical scores were based on the magnitude of paw edema. The mean temperature of the hind feet (region covering the metatarsus and tarsus) was normalized with a reference area on the back of the same rat. The temperature index were compared with the clinical score index, edema index, and bodyweight of the rats
Results: The mean hind feet temperatures increased with increasing clinical severity in the acute stage of the disease. There were positive correlation between temperature and clinical scores.

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Conclusion: The thermographic response appeared prior to the clinical signs, suggesting that thermography may be used as a predictive sign for the development of disease. This technique could be a non-invasive, objective, rapid, and reproducible method for evaluation of the degree of inflammation and effect of therapeutic interventions.

Keywords: Arthritis; collagen Induced arthritis; infrared; Lewis rat; non-invasive method; thermography;

1. INTRODUCTION

Rheumatoid arthritis (RA) in humans is a complex inflammatory multisystem autoimmune disorder. It commonly affects the joints in a polyarticular manner (polyarthritis). Collagen induced arthritis (CIA) in rats is a commonly used model for rheumatoid arthritis, however in view of the welfare of animals, improvements and refinements of these experimental models should always be considered (Griffiths, 1988; Trentham et al., 1977). Traditionally evaluation of the degree of inflammation in models of arthritis is based on histopathology. Histopathology gives information about the number of inflammatory cells at fixed time points, however the methods gives no information about the activity of these cells and the method excludes longitudinal studies. For longitudinal studies non-invasive methods such as magnetic resonance imaging (MRI) and positron emission tomography (PET) have been applied, however both methods are expensive and dependent on highly sophisticated equipment (Goebel et al., 2010; Irmler et al., 2010). This study evaluates the use of thermography, which is a non-invasive technique for measuring natural thermal radiation from the body surface, in a rat CIA model. It was hypothesized that the temperature of the affected area (hind feet) would correlate with the clinical score of the arthritis, and this technique could be utilized as an unbiased, rapid, non-invasive and reproducible method for quantification of the degree of inflammation and the effects of therapy.

2. MATERIALS AND METHODS

2.1 Animals

Sixteen adult female Lewis rats (LEW/Han™Hsd) from Harlan Laboratories weighing approximately 160-187 g were used. The experimental protocol was approved by The Danish Experimental Animal Inspectorate. The rats were housed (2-3 per cage) in a temperature-controlled (20±2°C) and lit (lights on 08:00–20:00h) room. Food (Altromin 1314) and water were freely available. At the end of the study the animals were anaesthetized with isoflurane, euthanized by cervical dislocation and thymus and spleen were removed, weighed and the relative wet weight of the organs ($W_{relative} = W_{organ}/W_{body}$) was calculated.

2.2 Induction of Collagen-induced Arthritis

Collagen induced arthritis was induced in the sixteen rats by injection of Immunization Grade Porcine Type II collagen (CII) (Chondrex cat. #20031) using a standard protocol (Griffiths 1988; Trentham et al., 1977). Porcine collagen type II (2 mg/ml) was dissolved in 0.05 M acetic acid by gently stirring overnight at 4 °C. Equal amounts of collagen (2 mg/ml) and

Incomplete Freund's Adjuvant (IFA) (Chondrex cat. #7002) were mixed in an ice-water bath, adding the collagen drop-wise to the IFA while mixing. Mixing was continued until a stiff white emulsion was evident and was able to congeal rather than dissipate when dropped in water. The arthritis-inducing emulsion was prepared immediately before immunization using an electric homogenizer. Rats were immunized with 0.2 ml (200 µg) of the emulsion by subcutaneous (SC) injection at the base of the tail under isoflurane anesthesia (Forene, Abbott). To ensure a high incidence and severity of arthritis a booster injection with 0.1 ml (100 µg) emulsion was given 7 days after immunization. The rats were divided in two groups. A vehicle group (12 rats): were treated with sterile phosphate-buffered saline, PBS, and a control group of four rats were treated with dexamethasone (1 mg/kg i.v.). Animals were treated 2 times per week for 2 weeks at the same days as the measurements were performed. All sixteen animals were measured before immunization and the data were included as not treated intact group of animals.

2.3 Temperature Assessment

The surface temperatures of the rats were measured while the animals were anaesthetized with isoflurane. An infrared video camera (SATIR-S280, GSAT INC.) was used to monitor the skin temperature pattern (local inflammation response in hind feet). The camera spectral range was 7.5-13 µm, which could measure temperature in the range of -40°C to +160°C to an accuracy of ±0.2°C. Temperatures on the hind feet (metatarsal and tarsus) were normalized with the shaved reference area on the back to be sure that the general temperature rise in the rat's body, due to collagen immunization, did not affect the measured difference in temperature rise in the hind feet. The mean temperatures in each hind foot (left: t_{left}, right: t_{right}) were normalized to the reference temperatures on the backs (t_{back}) of each rat; and the Temperature Index was calculated, (TI) ($TI = t_{left} / t_{back} + t_{right} / t_{back}$). Normal hind foot temperature in the intact group was approximately ≤26.55 °C (TI ≤ 1.5) while diseased hindfeet had a TI >1.5, where TI=1.5 corresponds to a temperature of 27 °C. Normal rat back temperatures were 36 $\pm$ 2 °C.

2.4 Clinical Score

We applied a standard clinical scoring system with 0 as unaffected and 4 as heavily affected joints with swelling (Table 1). Each hind foot was scored ($score_{left}$, $score_{right}$), and a Score Index calculated ($SI = score_{left} + score_{right}$).

Table 1: Interpretation of clinical scores in Lewis rats with collagen induced arthritis

Score	Clinical findings
	Normal
0.5	Light redness of ankle or digits
1	Mild, but definite redness and swelling of the ankle, or apparent redness and swelling limited to individual digits, regardless of the number of affected digits
2	Moderate redness and swelling of ankle
3	Severe redness and swelling of the entire paw including digits
4	Maximally inflamed limb with involvement of multiple joints

2.5 Measurement of Edema

Edema of the hindfoot was measured using a digital caliper (AccuGauge by DiLab, Lund Sweden), and was determined by calculating the cross sectional area (in mm^2) of the paw and ankle in each hindfoot ((Paw: A_p , Ankle: A_a). Each of these areas was normalized to the same hindfoot measured before the onset of treatment ($A_{normal\ p}$, $A_{normal\ a}$) and an Edema Index was calculated (A_p/ $A_{normal\ p}$, A_a/ $A_{normal\ a}$)). The overall EI was determined by taking the average of the sum of EI_{left} and EI_{right} ($EI = (EI_{left} + EI_{right})/2$). To calculate the area of the cross section (elliptical shape) of the paw and ankle two measurements were made for each section; paw: from side-to-side (a) and from top-to-bottom (b); ankle: from side-to-side and from front to-back at a 45° angle (Earp et al., 2009). The ellipse area was calculated by: *Area*= π. (*a*/2) . (*b*/2). The body weights (*W* in grams) of the rats were measured along with clinical scores, temperature and edema before any treatments on day 0 (intact) and on treatment days 7, 9, 11, 14, 17, 21,and 23 (endpoint).

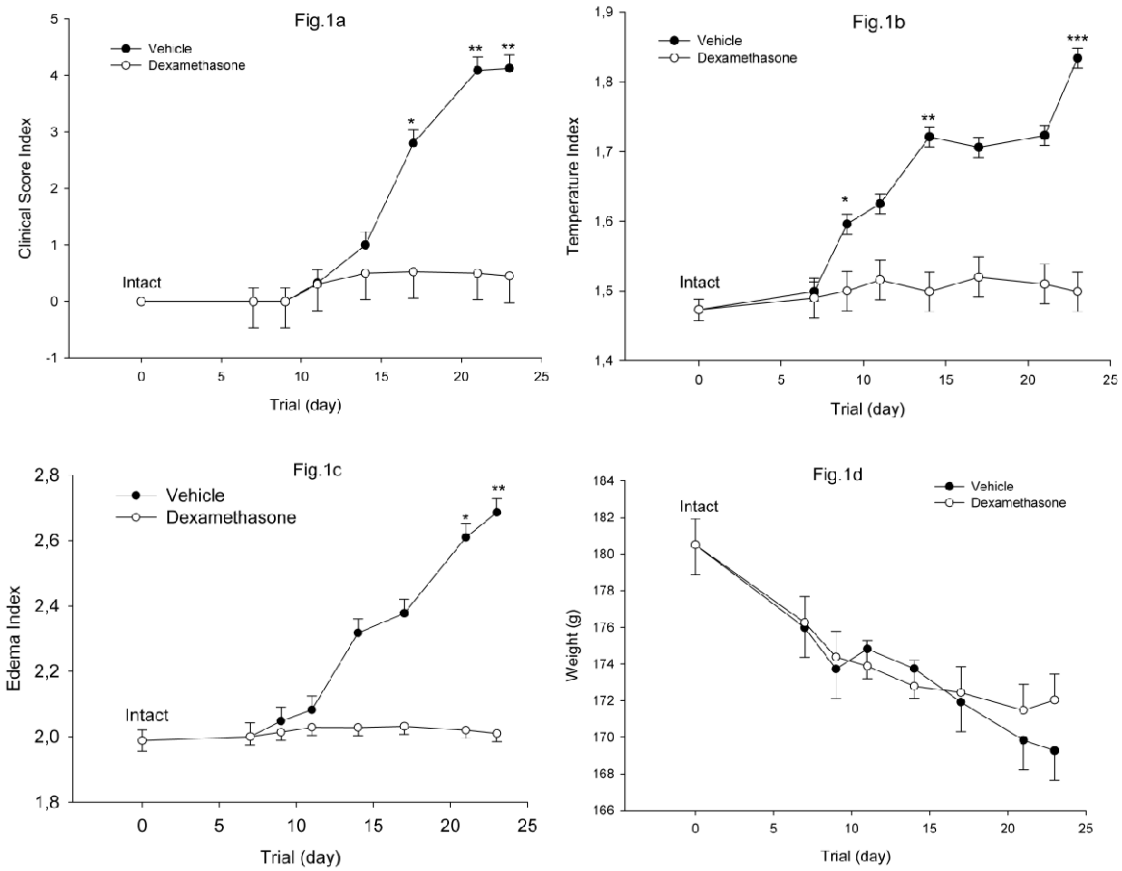

Figure 1: Comparision of clinical score, temperature, edema and weight between vehicle and dexamethasone groups

The mentioned parameters measured on day 0 (intact) and on day 7, 9, 11, 14, 17, 21 and 23 after first immunisation; a: Clinical score index (SI): P^*= 0.011 and P^{**}<0.001, b: Temperature index (TI): P^*, $P^{**}$and P^{***}<0.001, c: Edema index (EI): $P^*$and P^{**}<0.001 for hind feet in Lewis rats with collagen induced arthritis and d: Weight of the rats. There is no significant difference in weight reduction between dexamethasone and vehicle groups.

All values were expressed as a mean + standard error of the mean (s.e.m.). Comparisons of TI, SI, EI and W, were assessed applying ANOVA for two way repeated measures using SigmaPlot software version 11.0 both pair-wise and at different time points. Unpaired-t-test and Mann-Whitney Rank Sum Tests were used for comparisons of relative organ weight. *P* values < 0.05 were considered to be statistically significant.

3. RESULTS

As expected the rats in the vehicle group (fig.1a-c and 2a) developed arthritis while dexamethasone treated rats did not develop symptoms or only mild symptoms (fig.1a-c and 2b). As shown in fig.1a, there was no difference in clinical score between groups before day 17, whereas a significant increase in temperature on day 9 was observed comparing vehicle versus dexamethasone (diff. of mean= 0.118, $p<0.011$) see figure 1b. Temperature index 0.118 corresponds to 0.95 °C. The onset of the significant difference in the clinical score was seen on day 17 (dexa vs. vehicle: diff. of mean= 2.30, $p=0.011$). Generally a significant change in edema was observed. However the most significant change in edema was observed on day 21 (vehicle vs. dexamethasone: diff of mean 0.621, $p<0.001$) see figure1c. Comparing vehicle and dexamethasone groups with the intact group, both vehicle (diff of mean 14.771 g, $p<0.001$) and dexamethasone (diff of mean 13.331 g, $p=0.004$) groups showed a significant weight reduction. However, no significant difference in weight reduction was observed comparing dexamethasone with the vehicle group (fig.1d). As it was expected, the relative weight of the spleen (Diff. of Mean: 0.128 g, $p=0.035$) and thymus (Diff. of Median: 0.137 g, $p=0.029$) were reduced in the dexamethasone treated animals compared to vehicle treated. However no significant difference in relative weight of the liver was observed between vehicle and dexamethasone groups. Examples of thermographic images in a vehicle treated rat at different time points are seen in figure 3, where the score of the hind feet is also indicated (fig. 3).

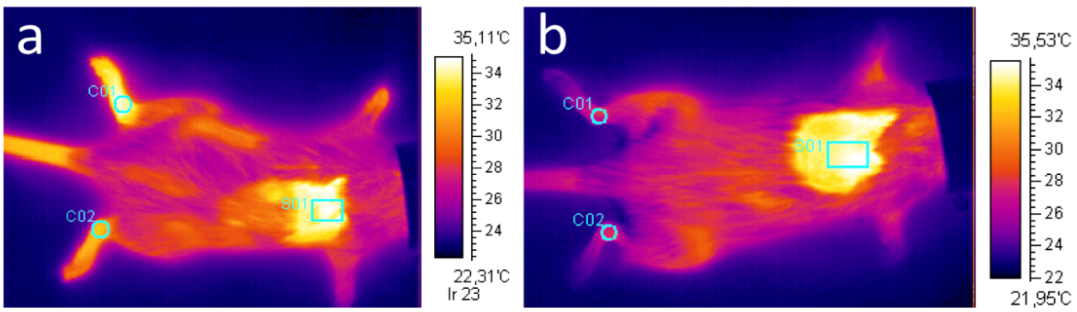

Figure 2: Two examples of thermographic images obtained with the infrared camera used in this study

The photos show a vehicle treated (a) and a dexamethasone treated rat (b) at the endpoint (day 23). As indicated by the color there is obvious temperature differences in the two hind feet, but also tail and the forepaw of the two animals have different temperature. The shaved reference area on the back of the animals is seen as a most bright area.

The study showed a strong correlation between increase in temperature and clinical score of the hind feet. This relation has a sigmoid pattern (fig. 4a). Mean temperature (day 7- day 23) on the hind feet was 30.29 °C in the vehicle group and 26.71 °C in the dexamethasone group. The temperature on the shaved area is seen in figure 4b. As indicated in the figure 4b, immunization with collagen induced a rise in the body surface temperature of the rats. The mean body surface temperature (day 7- day 23) measured on the shaved reference

area in vehicle group was 36.134 °C (95% C.I. of Mean: 0.224 °C) and in dexamethasone group was 36.07 °C (95% C.I. of Mean: 0.303 °C), however there was no significant difference between the two means. The body surface temperature on the shaved reference area in the intact animals was significant lower compared with same area in the vehicle and dexamethasone groups. It was 1.06 °C (P< 0.001) less compared to the vehicle group, and 1.12°C (P< 0.001) less compared to the dexamethasone group.

4. DISCUSSION

Thermographic studies have been used to evaluate inflammatory diseases of the hand (Devereaux et al., 1985) and assess osteoarthritis of the knees and hands (Dieppe et al., 1980; Ring et al., 1981) and the temperomandibular joint (Sanchez et al., 2008) in humans. Pain and stiffness have been shown to be significantly correlated with joint surface temperature in subjects with rheumatoid arthritis (Devereaux et al., 1985).

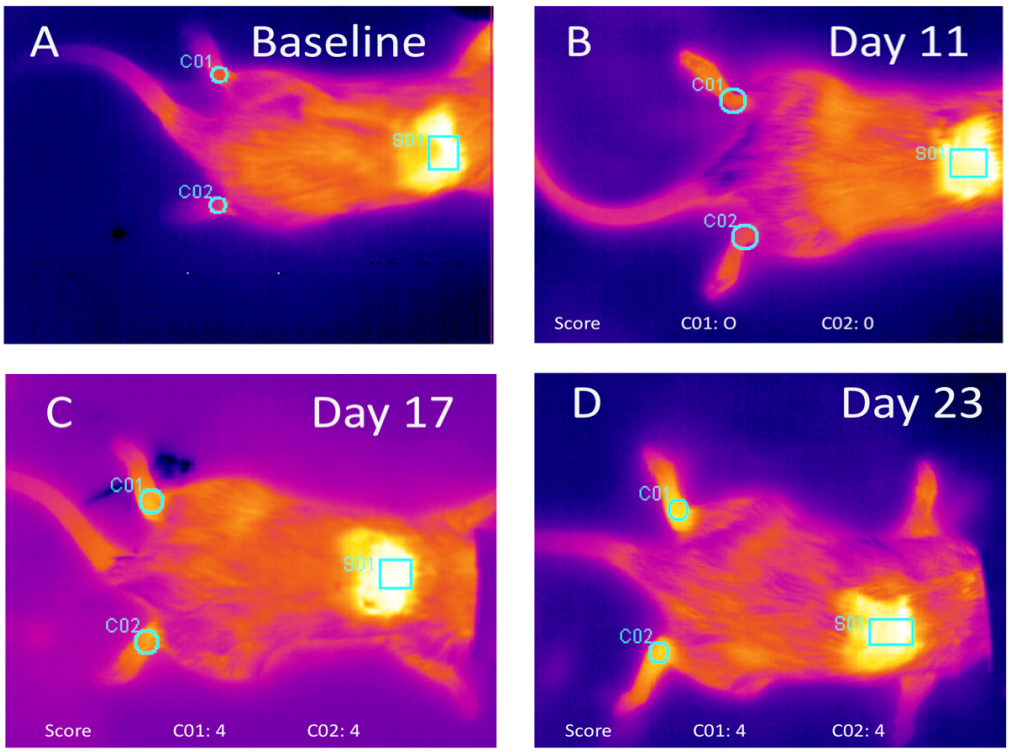

Figure 3: Infrared photos of one rat in the vehicle treated group at different time points before and after the development of the disease

Infrared photos of one rat in the vehicle treated group at different time points A: day 0, B: day 11, C: day 17, D: day 23. The figure further illustrates that hind feet may have same clinical score (C and D), while the temperature in the region of interest (ROI) is different as indicated by the differences in colors.

Thermography has been used as a tool to provide unbiased and reproducible data on joint inflammation in response to therapeutic interventions; for example, in the measurement of changes in hand joint temperatures in response to non-steroidal anti-inflammatory agents (NSAIDs) (Ring et al., 1981). Significant reductions in joint surface temperatures also occur

in knee arthritis 1 week after intra-articular steroid injections (Dieppe et al., 1980).

Sanchez et al. conducted a study of clinical scores in a CIA rat model (Sanchez et al., 2008) using a hand-held infrared thermography, similar to our own study, however they used higher doses of collagen and did not include a (dexamethasone) control group or body temperature reference (shaved back) areas; we observed a general body temperature rise in our CIA rats, and rises in joint temperatures could only be evaluated by standardizing our findings to these whole body reference areas. We also measured other parameters such as edema and organ weights to further verify that thermography was a reliable method of assessing inflammatory arthritis in this animal model. Thus it is difficult to make data comparisons with the Sanchez paper.

Brenner et al. (2006) also used thermal imaging technique to evaluate and monitor inflammatory arthritis activity in rat models, however they were not able to detect thermal changes before the onset of clinical disease in a systemic autoimmune model of arthritis induced with pristane (pristane induced arthritis; PIA).

Although our study demonstrated the feasibility of thermographic measurements, our rats were not monitored every day, leaving some data gaps. The inflammatory process is very dynamic and changes in the inflammatory stages may vary from day to day; thus it is difficult to follow the precise disease development in each joint of each study animal.

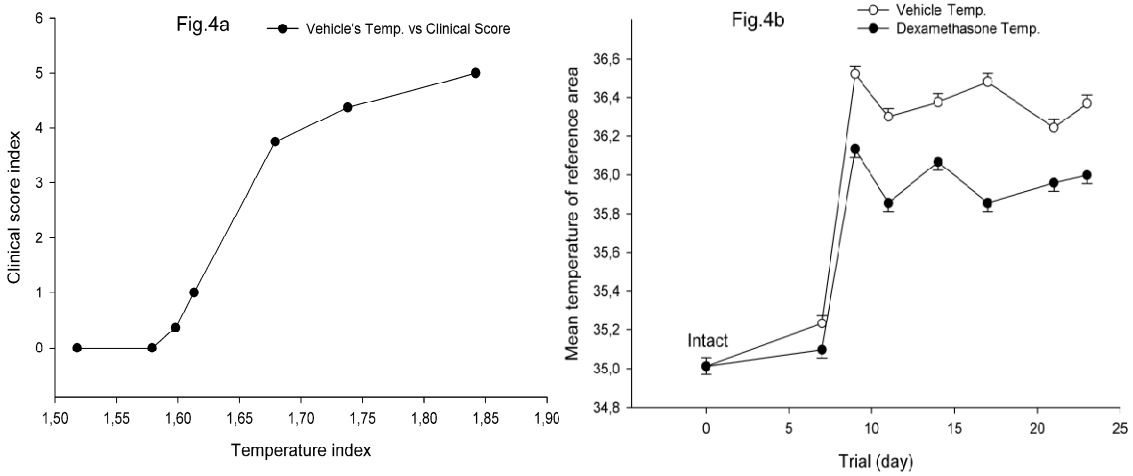

Figure (4a): Correlation between temperature and clinical scores in the vehicle group. (4b): Mean temperatures of the reference areas on the backs of rats treated with dexamethasone and in the vehicle group

Unfortunately histopathology was not performed in this study. Due to the limited number of animals only endpoint evaluation could have been obtained, not adding further information to the study. Histopathology requires sacrificing of the animal and therefore each animal will only contribute with a single snapshot and illustrate the number of inflammatory cells and tissue changes. Thermography on the other hand gives an indication of active inflammatory activity in the cells and tissue.

5. CONCLUSION

CIA rat models developed increased relative temperatures in their affected hind feet, which was inhibited by dexamethasone treatment. There was a strong correlation between an increase in relative hind foot temperature and the clinical score. Correlations between temperature and edema, and between temperature and loss of weight in these rats, support the application of thermography in the assessment of arthritis. The data indicates that a thermographic response may be observed even before the appearance of clinical signs, and thus thermography could be used as a predictive sign during the development of RA disease. This in turn might mean that therapeutic intervention is possible at an earlier disease stage due to an earlier diagnosis. Since it is possible to do repeated measurements not only numbers of animals can be markedly reduced, but it might also be possible to register the dynamics in longitudinal studies, as for instance monitoring of individual flare-up reaction or therapy response.

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