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Effects of exogenous deoxyribonuclease I in collagen antibody-induced arthritis

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Abstract

Background Rheumatoid arthritis (RA) is associated with a high concentration of extracellular DNA (ecDNA). This could be a consequence of the inflammation, but the ecDNA could also be involved in the unknown etiopathogenesis of RA. Clearance of ecDNA is hypothesized to prevent the development of RA. This study aimed to analyze the effects of exogenous deoxyribonuclease I (DNase I) administration in an animal model of RA.

Methods The collagen antibody-induced arthritis (CAIA) model of RA was induced in adult female DBA/1J mice. CAIA mice were treated with saline or DNase I (10 mg/kg) every 12 h for the whole duration of the experiment. Arthritic scores were assessed. Paw volume and temperature were assessed using a plethysmometer and a thermal camera, respectively. Plasma ecDNA and its subcellular origin were analyzed using fluorometry and real-time PCR. DNase activity was quantified with single radial enzyme diffusion method.

Results The CAIA model was successfully induced as proved by a higher volume, temperature and the overall arthritis score in comparison to controls. The administration of DNase I resulted in a nearly two-fold increase in serum DNase activity. Still, it did affect neither plasma ecDNA, nor the arthritis score or other measures of joint inflammation.

Conclusion Our results suggest that exogenous DNase I does not prevent the development of CAIA in mice. Whether this is true for other animal models of arthritis or clinical RA requires further research. EcDNA does not seem to be involved in the pathogenesis of CAIA. Additional studies are also needed to elucidate the role of ecDNA in the development of RA, focusing especially on its origin and inhibition of ecDNA release.

Keywords Cell-free DNA, Deoxyribonuclease, Autoimmune disease, Chronic inflammation

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Background

Rheumatoid arthritis (RA) is a chronic, autoimmune, inflammatory disease affecting around 1% of the population and its etiopathogenesis is still unknown [1]. The lack of knowledge about the real causes of RA leads to the absence of causal treatment [2]. The currently used biological disease-modifying anti-rheumatic drugs are the most effective treatment of RA in history. Still, side effects and a group of non-responders among patients require a further search for causal interventions [3, 4].

Similarly, to other autoimmune diseases, a higher concentration of circulating extracellular DNA (ecDNA)



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has been reported in patients with RA [5]. The release of DNA from cells can result from apoptosis, pyroptosis, autophagy, or the formation of neutrophil extracellular traps (NETs) [6]. NETs contribute to the increased ecDNA in RA, but other NETs such as citrullinated histones, myeloperoxidase, neutrophil elastase and antimicrobial peptides may play a role in the inflammatory responses typical for RA [7–9]. The fact that ecDNA release is driven by multiple mechanisms makes therapy targeting difficult. Potential protection of ecDNA from nucleases by extracellular vesicles further adds to the complexity. This could pose a significant challenge in developing treatments aiming to degrade ecDNA [10, 11]. Regarding subcellular sources, ecDNA can originate from the nucleus (nuclear DNA - ncDNA) or from the mitochondria (mitochondrial DNA - mtDNA) which has a higher potential to activate immune cells. EcDNA can be recognized as a damage-associated molecular pattern that induces an inflammatory response [12]. EcDNA is also present in healthy individuals, but in low concentration due to rapid removal by nucleases - deoxyribonucleases (DNases) [13]. The results of our previous study have shown that in RA patients, ecDNA concentrations positively correlate with the clinical parameters of disease severity, such as disease activity score (DAS28) and C-reactive protein [14, 15]. On the other hand, elevated ecDNA in RA might be more than just another biomarker of ongoing inflammation. It could be involved in the etiopathogenesis of RA, representing a potential causal therapeutic target [16].

As ecDNA is cleaved by nucleases, high ecDNA concentrations could be explained not only by increased release of DNA from cells but alternatively also by an insufficient clearance by DNases caused by their impairment or by the presence of their inhibitors as increased DNase I activity has been observed as a concomitant process in conditions where there is increased cell death [17–20]. Clinically, DNase is safely and efficiently used to treat cystic fibrosis. DNase reduces the viscosity of the mucus by digesting ecDNA in the alveoli [21, 22]. We and others have previously shown that exogenous DNase has beneficial effects in animal models of sepsis [23], hepatorenal injury [24], thrombosis [25] or ischemic-reperfusion injury [26]. Little is known about the effects of exogenous DNase in RA or animal models of RA. A recent study has shown promising results for the local intraarticular application of DNase [27]. However, RA is a systemic inflammatory disease and requires a systemic approach. Therefore, the present study aimed to investigate the effects of exogenous intraperitoneally administered DNase I in the mouse model of collagen antibody-induced arthritis (CAIA). In the current

study DBA/1J female mice were used to reflect the higher prevalence of RA in women compared to men [1]. We hypothesized that the clearance of ecDNA will prevent or reduce the severity of the induced arthritis.

Methods

Animals and housing conditions

In this study, female DBA/1J mice (12 months old) were used (Velaz, Prague, Czech Republic). Animals were group-housed (2–3 per cage) in polycarbonate cages (36.5×20.5×14 cm) and kept under standard laboratory conditions (temperature 22±2 °C, humidity 55±10%, 12:12 light-dark cycle). Animals had *ad libitum* access to food (standard diet for mice Eppy-KMK20, Bruzovice, Czech Republic) and water.

Groups of animals and experimental design

Mice were divided into the following groups: control group – CTRL ($n=5$) was injected with a saline (B. Braun Melsungen AG, Germany), CAIA group ($n=5$) was injected with the mouse anti-type II collagen 5-clone monoclonal antibody cocktail (1.5 mg/mouse, Chondrex, Woodinville, WA, USA), recognizing the conserved epitopes of type II collagen. The last group of mice was the CAIA+DNase I group ($n=5$). This group was injected with a monoclonal antibody cocktail as the CAIA group and DNase I (10 mg/kg, Sigma-Aldrich, Taufkirchen, Germany). Lyophilized DNase I was dissolved in 0.9% saline.

On day 0, CAIA females were injected i.p. with an anti-type II collagen 5-clone monoclonal antibody cocktail (1.5 mg/mouse, Chondrex, Woodinville, USA) followed by an i.p. lipopolysaccharide (25 µg/mouse, Chondrex, Woodinville, USA) injection on day 3 of the experiment. To assess the therapeutic potential of DNase I, the CAIA+DNase I group of females received an i.p. injection of bovine DNase I (10 mg/kg, Sigma-Aldrich, Taufkirchen, Germany) every 12 h from the beginning till the end of the experiment on day 8.

Clinical assessment of the severity of arthritis

Mice were monitored every second day during the whole experiment. The severity of arthritis was scaled from 0 to 4 points for every paw separately. Scoring was performed based on the mouse arthritis scoring system where three types of joints were observed - interphalangeal, metacarpophalangeal, carpal and tarsal joints. The scoring system was as follows: score 0 - normal/physiologic state, score 1 - one of the above-mentioned joints has redness and swelling, score 2 - two joints have redness and swelling, score 3 - all three joint types have redness and swelling, score 4 - achievement of maximal redness and swelling of the entire paw [28].

Plethysmometry

A plethysmometer (Ugo Basile, Gemonio, Italy) was used to evaluate paw swelling. The plethysmometer comprised two interconnected, clear acrylic tubes filled with a solution. Acrylic tubes were connected to a decoder that digitally presented the volume displaced after the paws of mice were placed into the prepared conductive solution. The paw volume was calculated as an average from three measurements for every paw [29].

Assessment of body temperature and temperature of paws

To assess the body temperature and temperature of all four paws a factory-calibrated handheld thermal imaging camera was used (Teledyne FLIR-E64501, Wilsonville, OR, USA). The body temperature correlates with the ocular surface temperature of mice, therefore, in the present study, the body temperature of mice was measured from the ocular surface [30]. During all temperature measurements, the room temperature was 24 ± 1 °C (humidity: $55 \pm 10\%$), and the mice were handled by the same experimenter on a stable platform. The distance between the camera and the mouse was 20 cm. Three thermal images were recorded for each mouse at the same time of the day. The thermal images were analyzed using FLIR Tools software (FLIR Teledyne FLIR-E64501, Wilsonville, OR, USA). The ocular surface temperature was determined as an average of the maximum temperature of the left and right ocular surfaces. The final body temperature was identified as an average from 3 facial thermal images. To measure the temperature of paws, each paw was marked with an ellipse shape using marking tools within the software. The final temperature of the paws was calculated as an average of three thermal images of the paws [31].

Measurement of paw swelling using micro-computed tomography

After collection of front paws, paws were snap-frozen in liquid nitrogen and immediately stored in a -80 °C freezer. For more accurate verification of the extent of the swelling, micro-computed tomography (micro-CT) was used (IVIS[®] SpectrumCT, Caliper Life Sciences, Hopkinton, USA). During scanning, the paws were placed in the scanner with a long axis aligned with the axis of the scanner bed. Obtained scans were analyzed in RadiAnt DICOM Viewer (Medixant, Poznań, Poland). The swelling of tissue was quantified as total paw thickness and also paw thickness inferior to metatarsal bones. The total paw thickness, in mm, was calculated as the distance across the whole paw including the metatarsal bones, the inferior thickness of the paw was measured from the metatarsal bones to the end of the soft tissue (Fig. 2G).

Bioluminescence

For noninvasive detection of inflammation, the bioluminescence of luminol is modified by the reaction with myeloperoxidase products. The luminol solution was prepared by dissolving powdered luminol sodium salt (Carbosynth, Berkshire, United Kingdom) in phosphate-buffered saline at a concentration of 50 mg/ml. To monitor the inflammation, the luminol solution (200 mg/kg body weight) was applied i.p. to mice [15]. Mice were anesthetized using isoflurane (Vetpharma Animal Health, S.L, Barcelona, Spain) inhalation (3% isoflurane, 97% oxygen) in the sleeping chamber during the measurements. Ten minutes after the application of the luminol solution, the mouse was placed into the chamber of the system IVIS[®] SpectrumCT (PerkinElmer, Waltham, MA, USA). The exposure time was set to 5 min. After the measurements, the bioluminescence signal from paws was quantified in the software LivingImage (PerkinElmer, Waltham, MA, USA) using average radiance (photons/s/cm²/sr) [32].

Histology scoring

Paws were fixed in neutral buffered 4% formalin for 6 h before the decalcification. Bones were decalcified for 1 week in 14% EDTA, pH=7.2 at 4 °C. Sagittal sections were stained with hematoxylin and eosin and scored by a blinded observer [33]. The scoring system was as follows: inflammatory cells infiltrate 0 - no infiltrate detected, 1 - modest leukocyte infiltration in synovial tissue, no fluid leukocytes, 2 - moderate leukocyte infiltration in synovial tissue and fluid phase, with loss of synovial architecture, 3 - gross leukocyte infiltration in synovial membrane and fluid space, significant loss of synovial articular architecture. Synovial hyperplasia: 0 - no abnormalities detected, 1- synovial lining layer 2–4 cells thick, 2 - synovial lining layer 5 and more cells thick associated with a moderate expansion of the sub-lining layer, 3 - synovial lining layer 5 and more cells thick associated with significant expansion of the sub-lining layer zone and potentially with loss of synovial architecture. Erosion of cartilage and bone: 0 - no abnormalities, 1 - fibrillation of cartilage and/or mild erosive infiltration of periosteal and subchondral bone, nuclei intact within lacunae, 2 - moderate fibrillation and loss of cartilage and/or moderate erosive infiltration of periosteal and subchondral bone, 3 - a significant loss of cartilage and/or erosive infiltration of periosteal and subchondral bone, nuclei show apoptosis within lacunae across a wide area of cartilage/bone.

DNA isolation and quantification

To quantify the concentration of ecDNA blood samples were collected on the day of the sacrifice into tubes coated with lithium heparin (Microvette 500 LH, Sarstedt, Germany). To separate plasma, samples were

centrifuged at $1600 \times g$ for 10 min at 4°C . Plasma was centrifuged again at $16000 \times g$ for 10 min at 4°C [34]. The supernatant was used for ecDNA isolation using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Quantification of isolated DNA was made by the Qubit 3.0 fluorometer and Qubit dsDNA high-sensitivity assay (Thermo Fisher Scientific, Waltham, MA, USA). The subcellular origin of ecDNA was determined using real-time PCR on the Mastercycler Realplex 4 (Eppendorf, Hamburg, Germany). The master mix used for the PCR reaction was SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). The PCR program was following: one cycle of 98°C for 3 min, 40 cycles of 98°C for 15 s for denaturation, 60°C for 30 s for annealing, and 72°C for 30 s for an extension. The sequence of primers to quantify the ncDNA (F:5'-TGTCAGATATGTCC TTCAGCAAGG-3', R:5'-TGCTTAACTCTGCAGGCG TATG-3'), while for mtDNA we used primers with the sequence F:5'-CCCAGCTACTACCATCATTC AAGT-', R:5'-GATGGTTTGGGAGATTGGTTGATGT-3').

Measurement of serum DNase activity

For the determination of DNase activity in serum a single radial enzyme diffusion (SRED) method was used. DNA agarose gel (1%, 20 mM Tris-HCl, pH 7.5, 2 mM MgCl_2 , 2 mM CaCl_2) with DNA isolated from chicken livers (0.035 mg/ml per gel) was prepared. For the visualization GoodView Nucleic Acid Stain (SBS Genetech, Beijing, China) and for the calibration values the DNase set with RDD buffer (Qiagen, Hilden, Germany) were used. 1 μl of the DNase stock and the samples were used. Prepared samples and standards (1 μl) were pipetted into the gel. Following an overnight incubation (at 37°C in the dark) the gel was visualized using iBOX (Vision works LP Analysis Software, UVP, Upland, CA, USA). Radial enzyme diffusion was then calculated from the gel using the ImageJ software (NIH, Bethesda, MD, USA). The DNase activity was expressed in Kunitz units (K.u.) per ml of serum [35].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.1 software (La Jolla, San Diego, CA, USA). Based on the Shapiro-Wilk test for normality of distribution, parametric One-way and Two-way Analysis of Variance (ANOVA) or Friedman's test was used for respective parameters with a Bonferroni-corrected post-hoc t-test. P values lower than 0.05 were considered statistically significant. Data are presented as mean \pm standard deviation (SD).

Results

RA score

Arthritis score based on inflammation signs constantly increased in the groups receiving monoclonal antibodies leading to a difference between the CAIA groups and the CTRL group of mice. A significant difference in the score was detected on day 6 of the experiment 8.33 ± 5.65 vs. 0 for CAIA and CTRL, respectively ($t_{(5)}=3.616$, $p=0.046$ (Fig. 1B)). Similar scores were observed on day 8 ($t_{(5)}=5.918$, $p=0.006$). No significant differences in arthritis severity were found between the CAIA and CAIA+DNase I groups during the whole experiment.

Body temperature and paw inflammation

During the experiment, no significant differences were observed in body temperature between the studied groups ($F=0.1432$, $p=0.8345$) (Fig. 1D). On day 6 of the experiment, mice of the CAIA and CAIA+DNase I groups showed higher temperature of the front paws than the CTRL group (by 1.5°C , CAIA: $t_{(7,69)}=5.50$, $p=0.002$, CAIA+DNase I: $t_{(8,26)}=5.26$) (Fig. 2B). On day 8, only CAIA+DNase I females displayed significantly higher temperature of front paws by 1°C compared to CTRL females ($t_{(6,69)}=5.01$, $p=0.005$) (Fig. 2A and B). Regarding the back paws, CAIA mice vs. CTRL mice showed higher paw temperature by 1°C , ($t_{(9,24)}=3.07$, $p=0.039$) only on day 8. CAIA+DNase I females had warmer back paws compared to CTRL group on day 6 and day 8 of the experiment by 1°C (day 6: $t_{(9,93)}=3.32$, $p=0.023$, day 8: $t_{(8,93)}=3.61$ $p=0.017$) (Fig. 2A and Fig. B).

Plethysmometer measurements

To verify peripheral inflammation and quantify the swelling of paws (paw volume), plethysmometer measurements were conducted (Fig. 1E). On day 6 of the experiment, front paws in CAIA+DNase I showed swelling by 18% higher than the CTRL group ($t_{(6,84)}=3.28$, $p=0.042$) (Fig. 1E). On back paws we could observe the interactive effect of time ($F_{(2,07, 29.5)}=6.18$, $p=0.005$) and treatment ($F_{(2, 15)}=4.12$, $p=0.037$), nevertheless no significant differences were observed in the partial analysis of swelling between the groups (Fig. 1F).

Bioluminescence and micro-computed tomography

The extent of inflammation was measured by luminol-based bioluminescence imaging. Results showed more than 9 times higher signal of inflammation in CAIA mice in comparison to the CTRL group ($t_{(12)}=3.08$, $p=0.029$). In addition, CAIA+DNase I females showed 1.5 times lower extent of the inflammation than CAIA

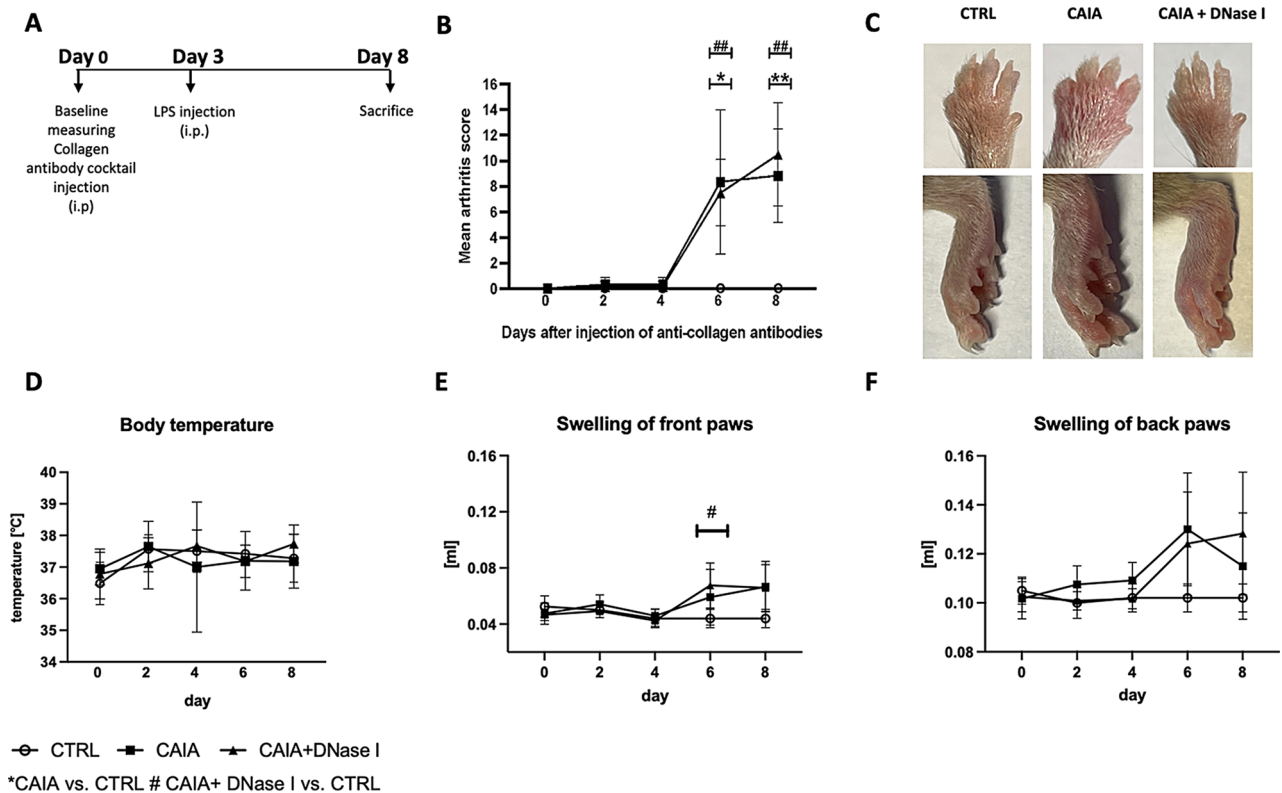


Fig. 1 Development of arthritis in mice. **A** timeline of the present study. **B** dynamics of arthritis score representing arthritis incidence and severity. **C** representative images of the severity of arthritis. **D** dynamics of body temperature during the experiment. **E, F** incidence of the severity of arthritis in the form of the swelling of front and back paws

mice however, these differences were not significant ($t_{(12)}=0.82$, $p>0.999$). Regarding the back paws, the luminol-based bioluminescence imaging showed no significant differences in the extent of the inflammation between the CAIA and CTRL females ($t_{(12)}=2.17$, $p=0.152$). The back paws of CAIA+DNase I showed a 5.25 higher bioluminescence signal than the CTRL group ($t_{(12)}=3.05$, $p=0.03$) (Fig. 2C and D).

The development of edema in the CAIA model was also monitored using the micro-CT (Fig. 2G and H). Total paw thickness (paw tissue with bones) was significantly higher by 25% on the front paws in CAIA+DNase I in comparison to CTRL mice ($t_{(14)}=3.75$, $p=0.007$). In the inferior part of the front paws, both, CAIA and CAIA+DNase I showed more than 2 times increased tissue volume than the healthy paws in CTRL group (CTRL vs. CAIA: $t_{(14)}=3.14$, $p=0.022$, CTRL vs. CAIA+DNase I: $t_{(14)}=3.29$, $p=0.016$) (Fig. 2G and H).

ecDNA and DNase activity

Plasma concentrations of ecDNA were not affected by the application of monoclonal antibodies against collagen type II ($F=0.54$, $p=0.59$) (Fig. 3A). Similarly, no differences were observed in the subcellular origin of ecDNA (ncDNA: $F=3.08$, $p=0.083$, mtDNA: $F=0.46$, $p=0.640$)

(Fig. 3C and D). Application of the exogenous DNase I led to higher DNase activity by 1.5 times between CAIA+DNase I and CAIA group, $t_{(13)}=4.19$, $p=0.003$, (Fig. 3D). There were no significant differences in DNase activity between CAIA and CTRL mice ($t_{(13)}=0.57$, $p>0.999$) (Fig. 3B).

Discussion

In this study we successfully induced the CAIA model in female mice. We tested the application of exogenous DNase as a possible intervention to prevent the development of arthritis. The administration of monoclonal antibodies led to inflammation - redness and swelling of the paws. Exogenous DNase, however, did not prevent the development of arthritis. No differences were seen in the arthritis score between the treated group and the mice with CAIA suggesting that ecDNA is not involved in the pathogenesis of this arthritis model.

In clinical studies, RA patients have higher plasma ecDNA in comparison to healthy individuals [5, 16]. EcDNA can be removed by endogenous and exogenous DNase [5, 16, 36]. The known pro-inflammatory properties of ecDNA support the rationale for inducing clearance of ecDNA that could mitigate inflammation [16, 37]. In our previous study, DNase administration improved

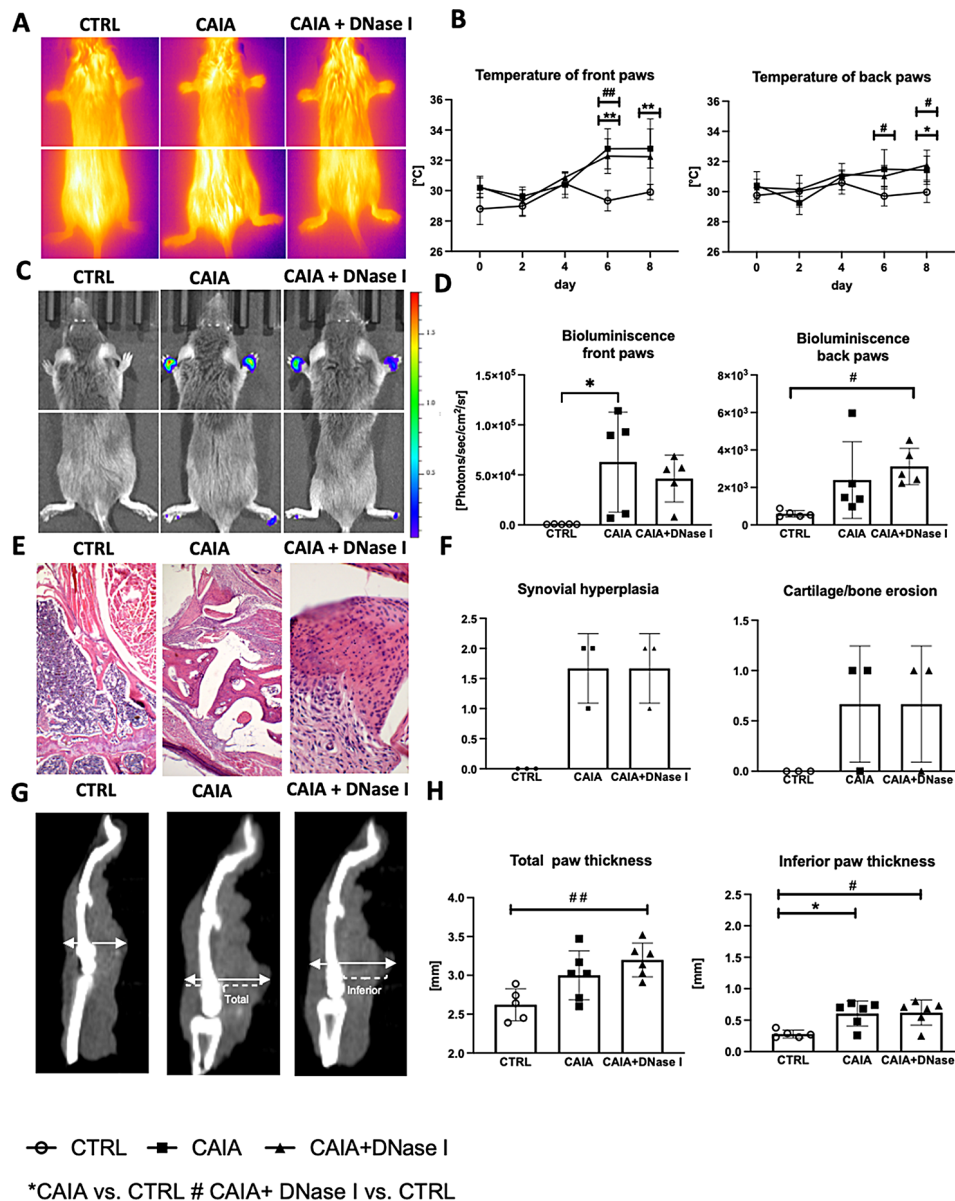


Fig. 2 Representation of arthritis signs at the end of the experiment. **A** representative image of the thermal camera pictures. **B** dynamics of the paw temperature. **C** representative images of the quantification of the inflammation-based bioluminescence. **D** dynamics of the paw bioluminescence. **E** representative images of the hematoxylin-eosin staining. **F** synovial hyperplasia and cartilage bone erosion. **G** representative pictures of the uCT, used for the assessment of tissue swelling. **H** representation of the swelling (total paw thickness and dorsal paw thickness)

survival in an animal model of sepsis [37]. In the present study, we were not able to reproduce the beneficial effect of DNase seen in another inflammatory model. A possible explanation could be the different route of application. In the model of sepsis, DNase was applied into the tail vein, in the present study DNase was applied intraperitoneally. However, the assessment of serum DNase activity confirms that the treated animals had higher DNase activity. So, the difference might rather be related to the disease models and their pathogenesis.

There are only few published studies on DNase in RA [27, 38, 39]. In the study by Zervou et al. polymorphisms

in the *DNASE1L3* gene were studied in association with autoimmune disorders such as RA [38]. In the animal experiment by Kawane et al. mice with a deletion in *Dnase2* gene suffered from chronic polyarthritis suggesting a similar cause in human patients with various types of arthritis [39]. Wang et al. in their study applied a hydrogel containing DNase to disrupt neutrophils extracellular traps to treat induced arthritis locally [27]. Only the latter one is an interventional experimental study. DNase fused with a hydrogel to increase its stability was applied locally to reduce arthritis in a mouse model [27]. This is an important difference to our study with systemic

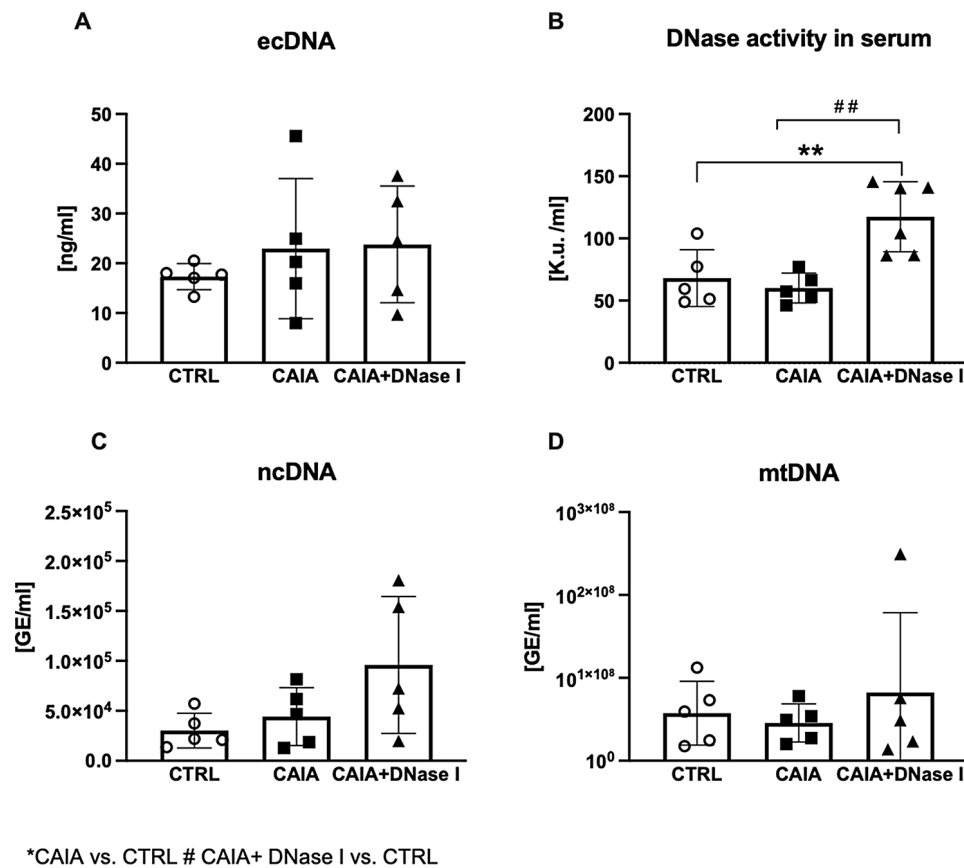


Fig. 3 Extracellular DNA (ecDNA) in plasma with its subcellular origin and deoxyribonuclease (DNase) activity. **A** concentration of total ecDNA in plasma. **B** DNase activity in serum after the application of the exogenous DNase. **C** plasma concentration of nuclear DNA (ncDNA), **D** plasma concentration of mitochondrial DNA (mtDNA)

application of DNase that is not stabilized and so, has to be applied repeatedly. Local administration might have a better local outcome, but RA is a systemic disease affecting many joints and with severe extraarticular manifestations. So, we believe that a systemic approach to treatment is necessary also in the CAIA model, which despite all the differences in the pathogenesis is still used as an animal model of RA. Wang et al. also used collagen-induced arthritis - a slower, chronic model. In our study, we used CAIA with acute inflammation and fast progress. It is, thus, clear that the next step should be the testing of exogenous systemic DNase in the collagen-induced arthritis as well.

A reason for the lack of effect of DNase in CAIA could be the lack of effect of CAIA on ecDNA. In contrast to RA in human patients, CAIA induction in mice did not increase ecDNA suggesting a difference in the pathogenesis and pointing towards a major limitation of this model for the research of RA. Despite clear signs of inflammation in mice, CAIA did not affect ecDNA. The reasons could include the multiple, but local inflammation in CAIA vs. systemic inflammation in RA, but also the different duration of the inflammation in mice vs. human

patients [1, 30]. In our study, the discrepancies regarding ecDNA concentrations might originate from the used experimental model. RA has a complex pathogenesis, making its modeling difficult. Consequently, various animal models of RA have been developed, each focusing on a specific aspect of the pathogenesis. No single animal model currently mimics the entire RA pathogenesis. Thus, ecDNA concentrations might vary between different models and the human disease [40]. We have studied the effect of DNase I treatment in collagen induced arthritis as this model was already established in our laboratory [41]. Unfortunately, we are not able to collect enough synovial fluid from mice to confirm higher local ecDNA and the potential effect of DNase on this ecDNA.

According to our knowledge, this is the first study describing the effect of intraperitoneal DNase administration in the CAIA model. Based on our results this new potential preventive treatment failed to affect the development of arthritis. Also, this is the first study to analyze ecDNA and its subcellular origin in the CAIA model. Again, our results suggest that ecDNA is not affected by CAIA and is not involved in its pathogenesis. However, due to differences between human disease and

its animal models, this does not mean that DNase cannot be useful for the prevention or treatment of RA. To prove this hypothesis other models and potential clinical trials are needed. The source of ecDNA in inflammation are mostly neutrophil extracellular traps and these are elevated in patients with RA [36]. Neutrophils have an important role in the progression of RA, but maybe also in its initiation. NETs are web-like structures with a DNA scaffold coated with proteins which could act as auto-antigens [42]. RA is a chronic inflammatory disease primarily affecting joints. Therefore, the research literature is focusing on the site of the inflammation and the neutrophils in the synovial fluid are investigated [43–46]. It has been reported that neutrophils in the synovial fluid show altered phenotype in RA resulting in higher NETs formation [43]. Furthermore, the presence of NETs in the synovial fluid was associated with osteoclastogenesis leading to bone loss and articular damage [47, 48]. In addition, NETs drive T cell differentiation via activation of Toll-like receptors by NETs-associated histones and nucleosomes [49]. It is of importance that NETs contain citrullinated proteins stimulating the formation of anti-citrullinated antibodies [32]. Therefore, citrullinated proteins represents a strong association between the NETs and RA with a bridge to local inflammation primarily affecting the musculoskeletal apparatus. However, why does the phenotype of neutrophils in the synovial fluid change and what could be the reasons for the increased formation of NETs is still unclear [43].

Studies investigating the pathogenesis of RA suggest that NETs production could lead to higher concentration of ecDNA, which further triggers the inflammatory process [14, 15, 50]. The release of ecDNA inside vesicles or at least associated with vesicles such as exosomes represents a challenge for the removal of ecDNA [51]. Histones, cathelicidin and other antimicrobial peptides likely protect ecDNA derived from NETs from degradation [52]. Thus, components of NETs could cause the low efficacy of DNase I application in the present study. NETs as the major source of ecDNA in inflammation influence the accessibility of DNA to DNase I [53]. Efficacy of DNase I could be also be altered by its inhibitors, for example anti-DNase antibodies. In addition, the presence of anti-NETs antibodies could contribute to lower dissembling of NETs as it was reported in patients with systemic lupus erythematosus [54, 55]. The application of DNase I has already been shown to have beneficial effects in mouse models of sepsis and hepatorenal failure [24, 37]. However, differences in the structure of ecDNA in various diseases and disease models could cause the variability of DNase effects. Whether extracellular traps with ecDNA as their main compound are also higher in animal models of RA is not clear. Despite negative outcomes, our study might help to focus further research on RA linked to

early biomarkers or more importantly to uncovering the unknown etiopathogenesis of RA. Despite the currently available biological therapy, there is still a large group of RA patients who would profit from a causal targeted treatment.

Conclusion

Application of the DNase I did not cause major and clinically relevant changes between the treated and the untreated groups of CAIA mice. Results from this study suggest that ecDNA is not involved in the pathogenesis of rheumatoid arthritis, however, this could be influenced by the selected animal model, which represents only, very acute, and local inflammation. EcDNA thus can be increased in the site of inflammation but within the laboratory rodents, we are limited with the extraction of the fluids. Therefore, new analysis with different animal models of rheumatoid arthritis should be performed to prove if the ecDNA stands behind rheumatoid arthritis.

Abbreviations

| | |
|----------|-------------------------------------|
| CAIA | Collagen antibody-induced arthritis |
| DAS28 | Disease activity score |
| DNase I | Deoxyribonuclease I |
| DNases | Deoxyribonucleases |
| ecDNA | Extracellular DNA |
| micro-CT | Micro-computed tomography |
| mtDNA | Mitochondrial DNA |
| ncDNA | Nuclear DNA |
| RA | Rheumatoid arthritis |

Author contributions

Authors contributions PC., B.V., E.Š contributed to study concept and design. K.M., V.B., M.T., J. J., and M.P. performed in vivo and in vitro experiments. R.H. and N. M., performed the histology analysis. K. M., V.B., and M.T. analyzed the results and made the figures. K.M., V.B., and P.C., wrote the final draft of the article. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analysed in the current study are available from the corresponding author upon reasonable request.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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