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Near-infrared fluorescence imaging with Indocyanine Green-Lactosome in the mouse model of Rheumatoid Arthritis.

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Figure

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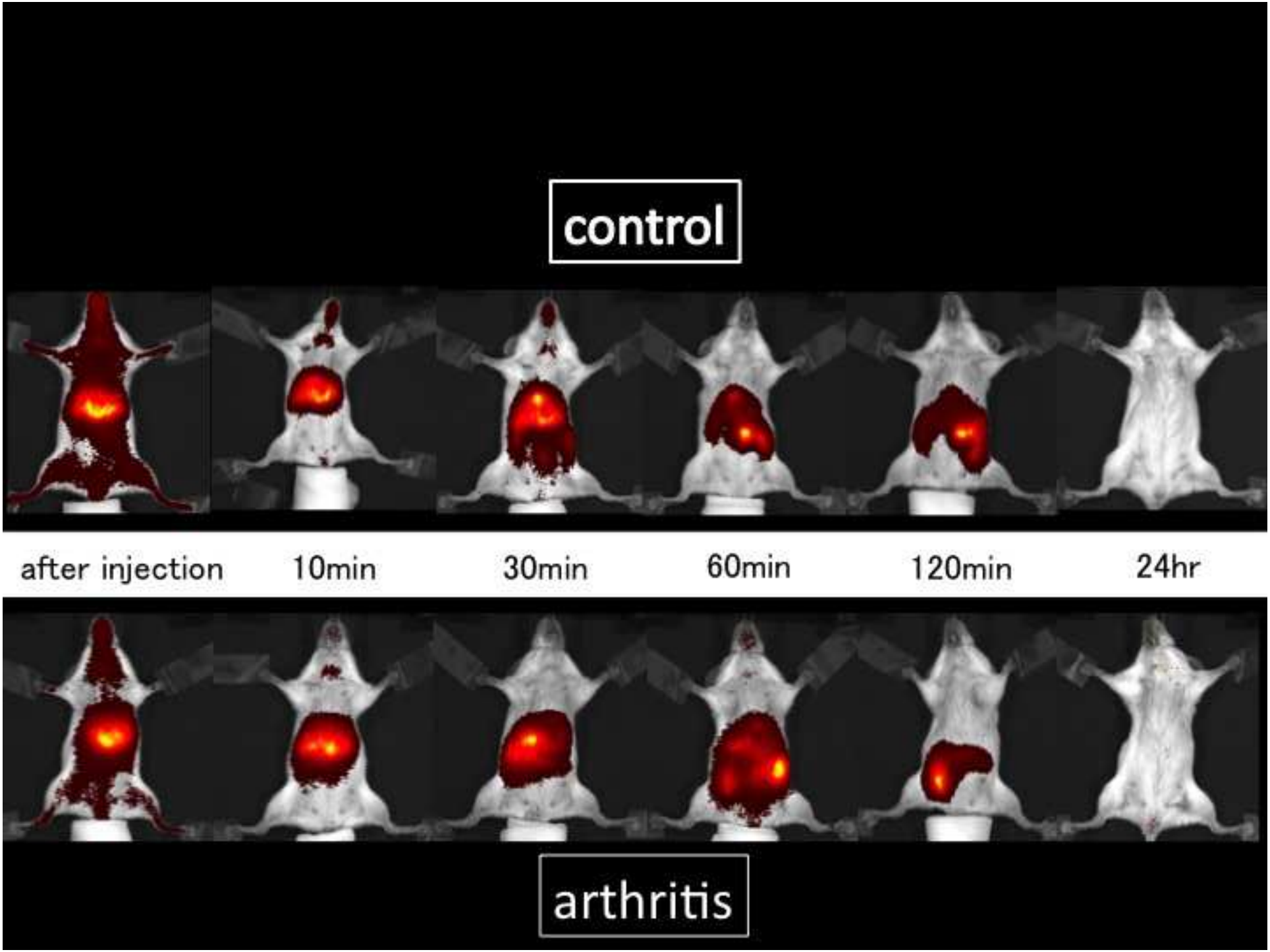

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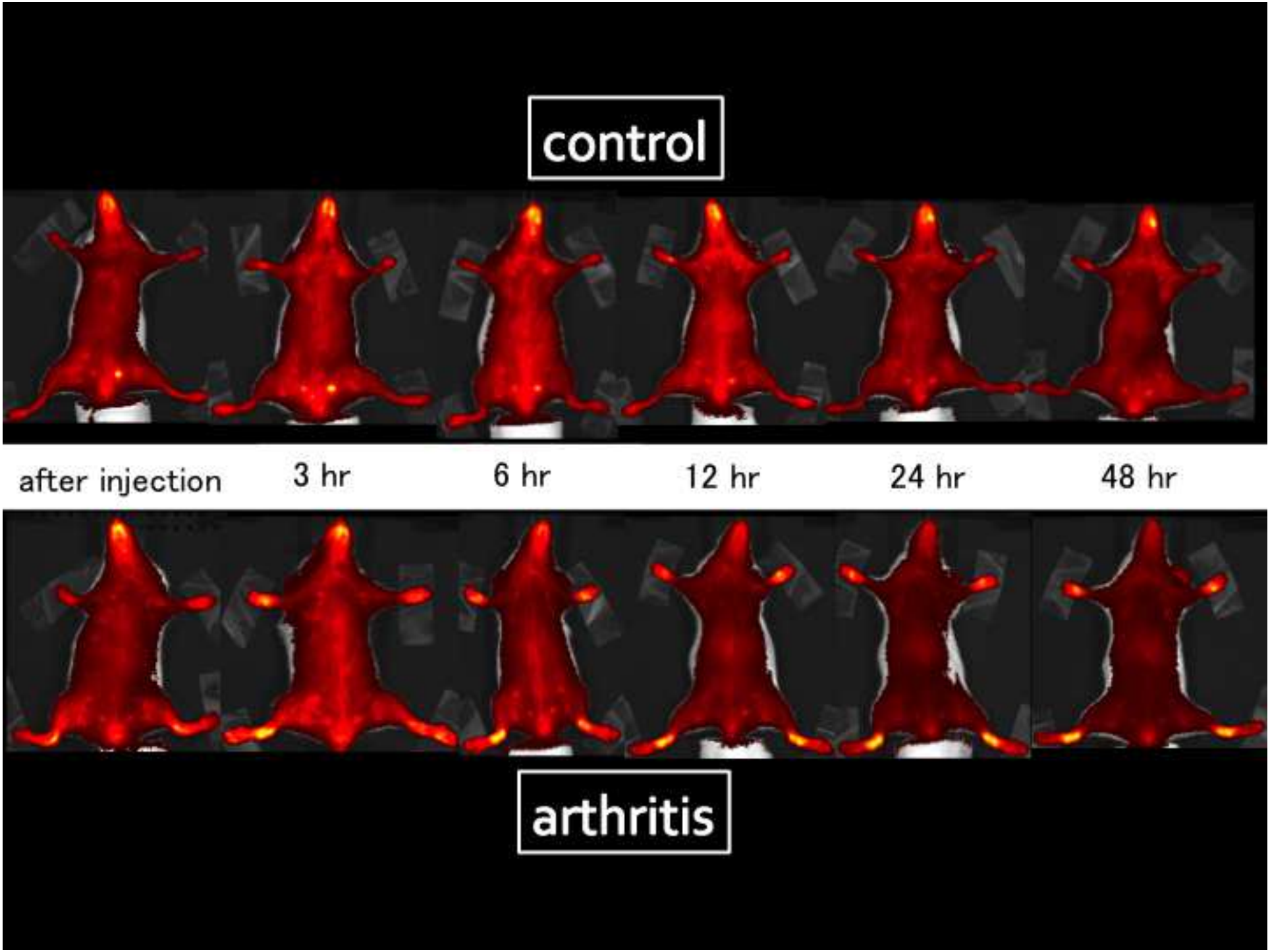

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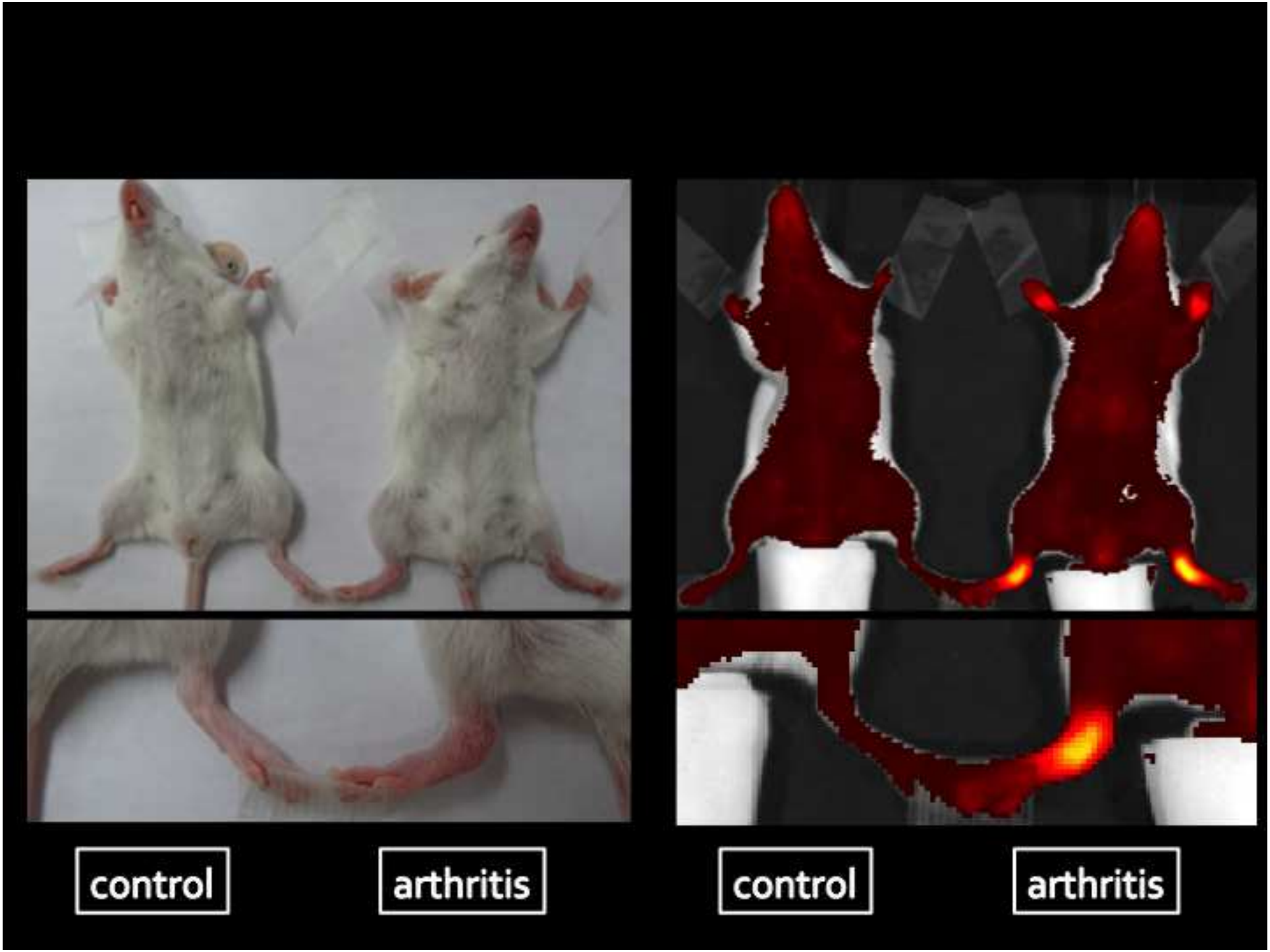

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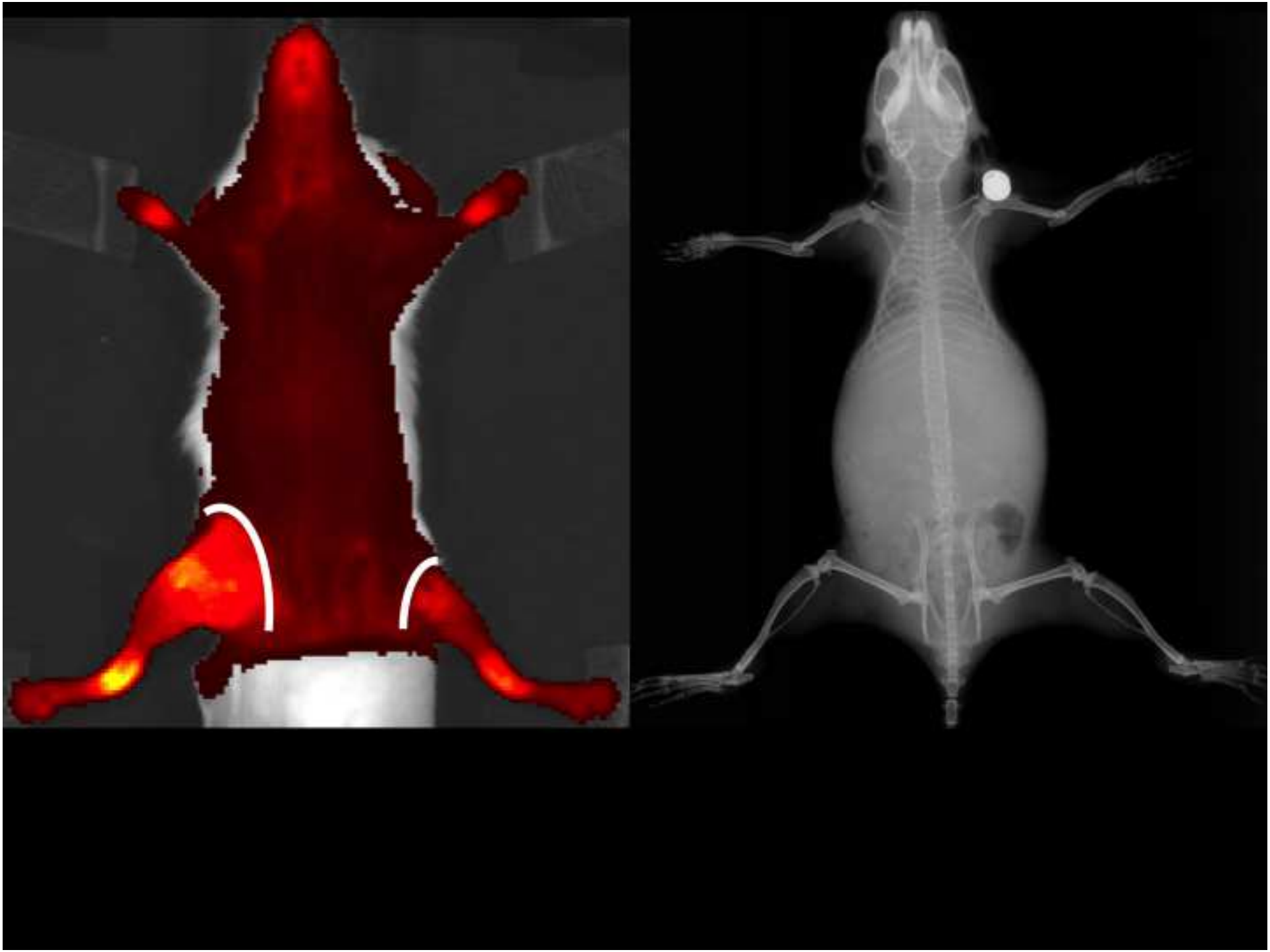

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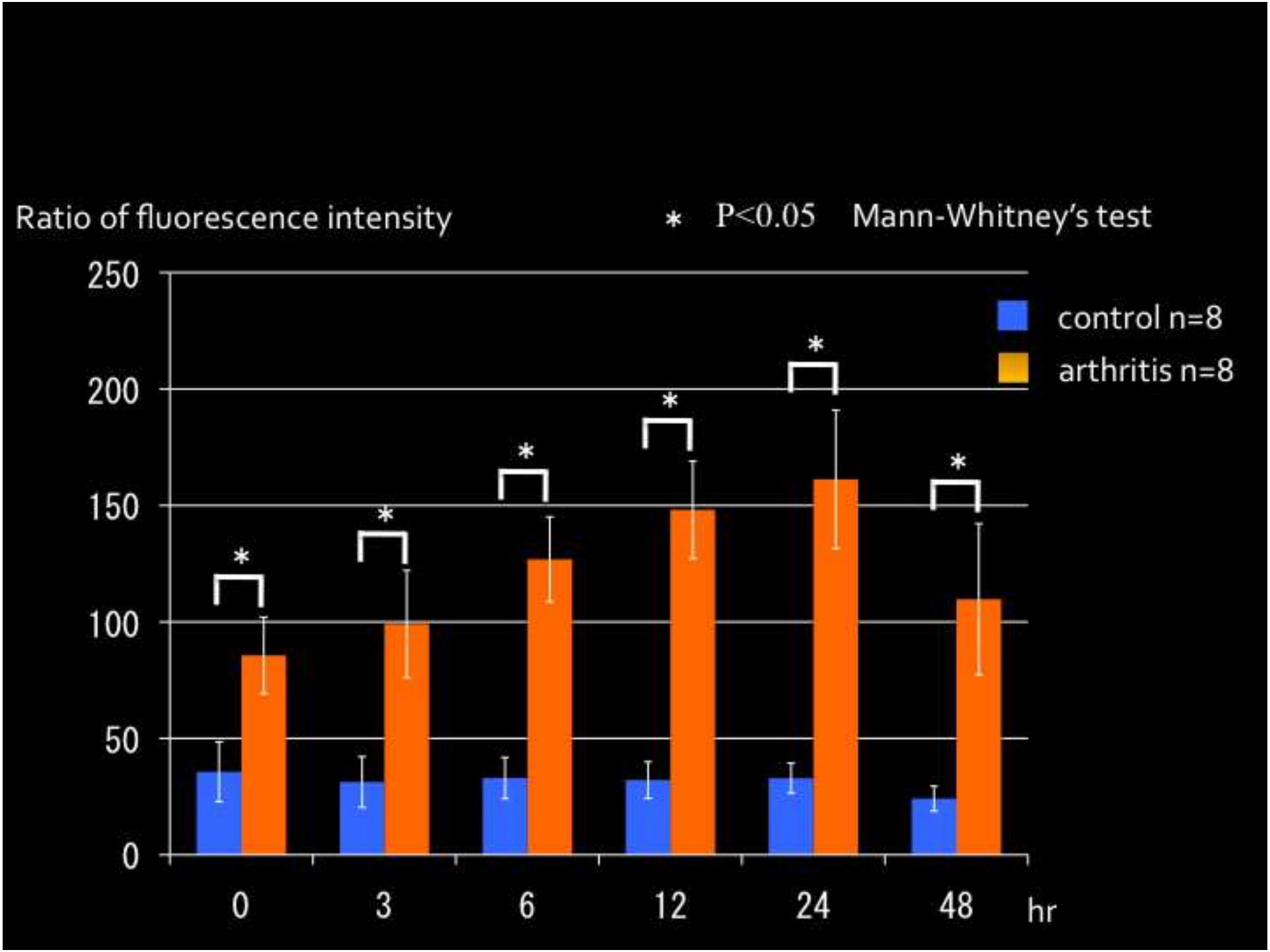

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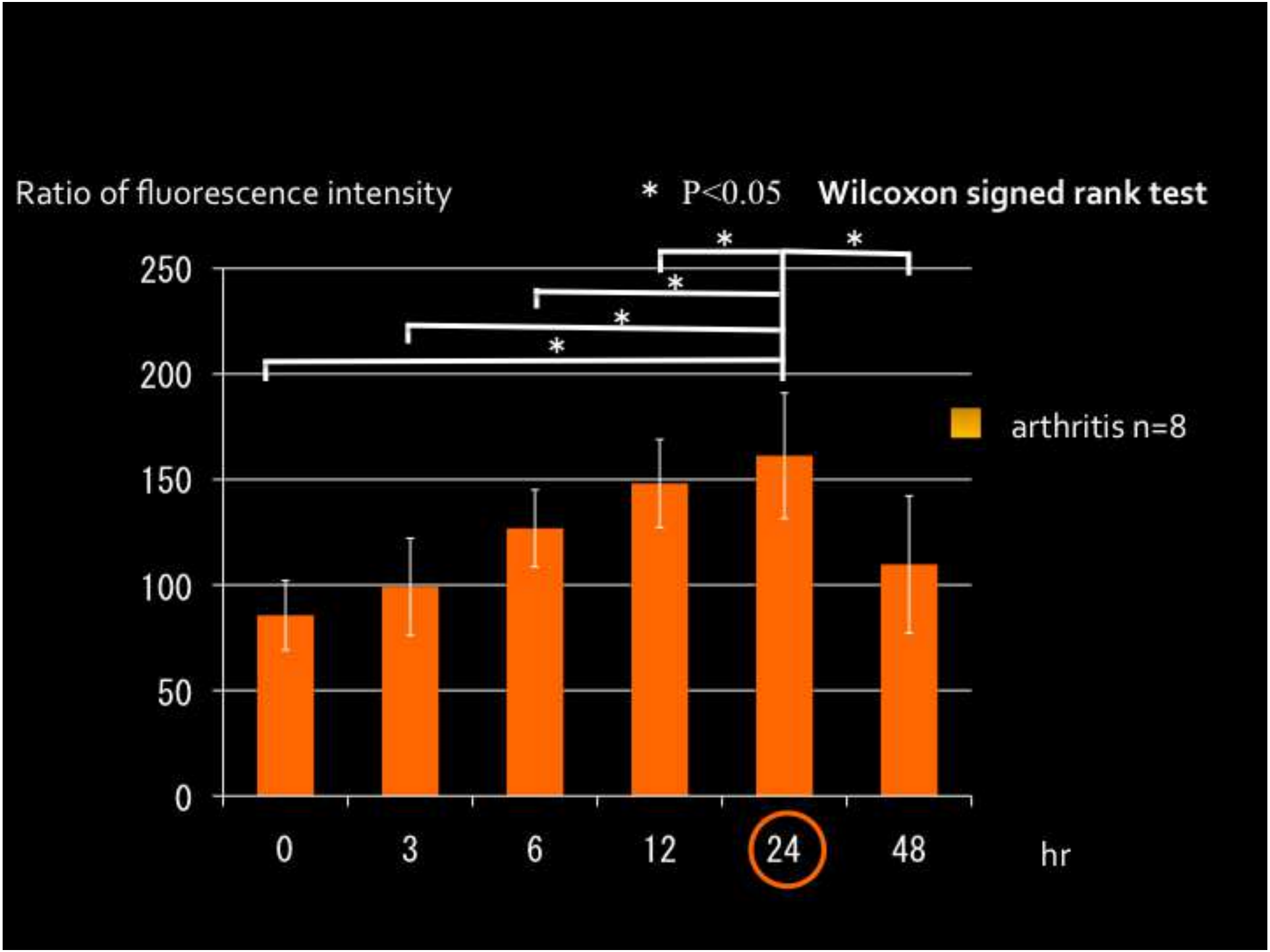

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Title: Near-infrared fluorescence imaging with Indocyanine Green-Lactosome in the mouse model of Rheumatoid Arthritis.

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Abstract

Objectives: Early diagnosis of Rheumatoid Arthritis (RA) is important to reduce the destruction of the joint. Current imaging have problems such as contrast agent, cost and time, and need for examination by specialists. Fluorescence imaging has been emerging as a new way of solving the problems. Our aim was to verify the fluorescence imaging using ICG-Lactosome and near-infrared light in the mouse model of RA.

Methods: We used the SKG/Jcl mouse. We took fluorescence imaging after ICG and ICG-Lactosome administration, and examined the fluorescence intensity ratio in the region of interest.

Results: In the ICG, fluorescence luminance decreased in a short time, the fluorescence intensity of the joints did not increase. Meanwhile, ICG-Lactosome was retained for a long time and the fluorescence luminance was rising in arthritic sites. The fluorescence intensity ratio was increased statistically significant, and its peak was 24 hours. In the arthritis of RA, enhancement of vascular permeability caused by cytokines, it was considered that ICG-Lactosome was leaked outside the blood vessel by the enhanced permeability and retention effect.

Conclusions: Near-infrared fluorescence imaging with ICG-Lactosome was possible to detect arthritis of mouse model of RA, we considered this has a reliable and simple method than the ICG.

Introduction

Rheumatoid arthritis (RA) is a chronic and progressive systemic autoimmune disease characterized by joint synovitis that causes destruction of bone and cartilage, finally made joint deformity. The occurrence rate of RA is 0.5-1.0% in several European and North-American populations. And the onset age of RA is 20-60 years old when their daily activity is very high. To prevent the joint destruction and improve the prognosis, early treatment is important because the joint destruction is seen in mostly within two years after onset. Biological therapy changed systemic treatment of RA dramatically, but it is still needed to treat local arthritis. X-ray, ultrasound, CT, MRI, PET, SPECT is used as the imaging of RA. Although some of these tests are useful for detecting arthritis early, but they have problems that need contrast agents, radiopharmaceuticals, time and cost, and interpretation by specialists.

Nowadays, it is seen some reports about the fluorescence imaging that use nanoparticle and light as new method of imaging. Enhanced permeability and retention (EPR) effect is a phenomenon that nanoparticles of diameter 10-200 nm leak from capillary vessels whose permeability is abnormally increasing and accumulate in the interstitial space in the rapidly growing tumor tissue [1]. Fluorescent labeled nanoparticles leak and accumulate by the EPR effect, imaging is being performed by irradiating with light. Near-infrared ray (NIR) whose wavelength is 600~1000 nm have good biological transparency, so an agent that emits near-infrared fluorescence can be visualized from the surface after it has been administered. ICG has an excitation wavelength 774 nm, fluorescent wavelength around 805 nm, and has little toxicity. But ICG is metabolized in the liver immediately after intravenous injection, so it has been difficult to take images without specialist.

Kyoto University and Shimadzu Corporation developed "Lactosome" consists of poly-sarcosine and poly-L-lactic acid (PS-PLLA). Lactosome is a lactic acid-based amphiphilic polypeptide polymer micelles, and it is safe because biodegradable. "ICG-Lactosome" is Lactosome labeled with ICG. ICG-Lactosome is nanoparticle of diameter 35 nm, leak by the EPR effect and stay long time in the body because it is not less recognized by the reticuloendothelial system (RES). Thus ICG-Lactosome made it possible to take images of tumor [2].

We hypothesized that, in the RA, vascular permeability is also enhanced by the inflammatory reactions and expected to the similar mechanisms of EPR effect on tumor. ICG-Lactosome is safe as mentioned above, and is considered to be applicable to the new RA arthritis evaluation. So our purpose of this study is to examine the near-infrared fluorescence imaging using ICG-Lactosome and the utility for ICG alone in model mice of RA.

Materials and Methods

RA Model Mouse

We observed the ankle joint of female SKG / Jcl mice (CLEA Japan, Inc., Tokyo, Japan) that develop the immune arthritis that closely resembles human rheumatoid arthritis. SKG / Jcl mice is in an autosomal recessive inheritance, has a point mutation of the ZAP-70 gene, and develops the joint swelling (female > male) in 2 months after birth. Immunologically, they have anti-RF IgM, anti-type II collagen antibody, antibody that reacts with Mycobacterium tuberculosis HSP70 in serum and leading to immunoglobulin glycosylation abnormalities with age. However, SKG / Jcl mice has a feature that it is difficult to develop arthritis spontaneously under specific pathogen free environment. So we developed arthritis by i.p. administration of mannan(Sigma-Aldrich, Co., St. Louis, MO, USA) 20mg at 8 weeks of age and reared six weeks in accordance with past literature [3]. The arthritis group is 4 animals 8 limbs (14 weeks old, onset of arthritis is 8 weeks old), and control group that did not develop arthritis is 4 animals 8 limbs (14 weeks old). We injected ICG-Lactosome 2.0 mg / body, and ICG as equal mol as ICG-Lactosome (4.0 nmol) into caudal vein.

Arthritis Score

In accordance with the past literature [4], we calculated macroscopic arthritis score of mice that made the ICG-Lactosome administration. Finger and toe count 0.1 point when the joint is swelling, and the wrist and ankle count 0.5 point when seen loss of constriction of Achilles tendon attachment portion and count 1.0 point when ankle joint is rounded.

Optical Imaging

In order to confirm the accumulation of the arthritis site of ICG and ICG-Lactosome, we carried out the fluorescence in vivo with imaging device（Xenogen IVIS Spectrum Living Image® Version4.3.1; PerkinElmer Inc., Waltham, MA, USA） set in excitation wavelength 745 nm and fluorescence wavelength 840 nm. Imaging after ICG injection into caudal vein did as soon as after injection, 10, 30, 60, 90, 120 minutes, and 24 hours. Imaging after ICG-Lactosome injection did as soon as after injection, and 3, 6, 12, 24, 48 hours.

Fluorescence Intensity Ratio

We set the region of interest (ROI) of circle of diameter 3.5 mm in the ankle joint. We calculated fluorescence intensity ratio by divided the ROI value of each imaging time by the ROI value of before ICG or ICG-Lactosome injection. The fluorescence intensity ratio of the control group and arthritic group at each imaging time were tested statistically by Mann-Whitney's test, and the fluorescence intensity ratio in arthritic group was tested by Wilcoxon signed rank sum test ($P<0.05$).

Results

Average of Arthritis Score

The arthritis score is shown in Figure 2. The average of arthritis group (n = 4) was 4.73 ± 0.62 and control group (n = 4) was 0. Individuals of Arthritic group were possible to confirm the onset of arthritis macroscopically.

Assessment of Optical Imaging

(1) ICG.

Both in arthritis group and control group promptly accumulated in the lever after 10 minutes and did not accumulated in the ankle joint. Then it was almost disappeared in 24 hours after administration (Fig. 1).

(2) ICG-Lactosome.

In arthritis group, the fluorescence luminance of the ankle joint was rising since after 3 hours, but there was no rise in the control group. Both in arthritis group and control group, systemic fluorescence luminance had been rising even in 48 hours (Fig. 2). In simultaneous imaging of an arthritis mouse and a control mouse at 24 hours after administration, only arthritis group showed fluorescence luminance enhancement (Fig.3). In the fluorescence imaging by IVIS, if the fluorescent material is closer to the body surface, the fluorescence luminance will rise more. Since the other joints are covered with hair, high luminance isn't observed. But we could observe the enhancement of fluorescence luminance of the knee joint when ablated the skin (Fig.4).

Analysis of Fluorescence Intensity Ratio

(1) ICG.

The maximum fluorescence intensity ratio was immediately after the administration in both arthritis and control group. And its fluorescence intensity ratio was less than 10 times.

(2) ICG-Lactosome.

Whereas the fluorescence intensity ratio of arthritis group was increased to up to 150 times near, the control group was less than 50 times. The arthritis group compared to control group, was significantly greater at each time (Fig. 5). In addition, in the arthritis group, the peak of fluorescence intensity ratio was at 24 hours after ICG-Lactosome administration, and the fluorescence intensity ratio was significantly greater than any other time (Fig. 6).

Discussion

In arthritis of RA model mouse, capillary permeability is enhanced by angiogenesis, increased blood flow in the growth synovium, cytokines like IL-6, IL-1-6, IL-1, IL-β, TNFα etc. In malignant tumors, nanoparticles have the EPR effect that leak out from immature walls of new blood vessels and retain in the outside of vessels. It has been used in molecular imaging and the Drug Delivery System of anticancer drugs [5, 6].

The diameter of the ICG-Lactosome is small as 35nm, and less likely to be metabolized in the kidney and liver for the micelle surface is covered by hydrophilic poly-sarcosine. In the arthritis, ICG-Lactosome leak into the vessel stromal space by the EPR effect that is same way in tumor, and is retained by not recognized by the RES, it is considered that the fluorescence intensity was enhanced. The blood half-life of ICG-Lactosome in mice is reported as 17.8 hours [7], so in this present study, the fluorescence intensity ratio in the arthritis group considered to be peaked at 24 hours after ICG-Lactosome administration. However, there is a need for further verification whether it is same results as RA in other arthritis disease.

In the study that performed imaging of ICG alone in the hand of healthy people and RA patients, reported that could distinguish the healthy joints and arthritis [8, 9]. It is also reported that could detect early arthritis and evaluate the therapeutic effect [9, 10]. Compared to MRI, it can perform without contrast agent that may occur side effect, less consumption of cost and time, and the inspection unit is simple. But it takes an ROI measurement and professional judgment.

In the imaging with ICG-Lactosome, the diagnosis is easy because we can catch arthritis joint more clearly in visual and can raise detection capability without professional judgment compared with imaging of ICG alone. In this study, we could detect the developed arthritis. And it is further challenge whether it can be detect initial change. Further more, after recognize the initial arthritis, if it is

possible to excite the ICG-Lactosome by irradiate with near-infrared laser and suppress synovitis by singlet oxygen and cytotoxic decomposition agents caused by the photodynamic reaction, there is a possibility that allows diagnosis and treatment at the same time.

Conclusion

The arthritis of the RA model mouse was able to detect by near-infrared fluorescence imaging using ICG-Lactosome, and the optimum time was 24 hours after the ICG-Laactosome administration when the difference between the normal tissues was maximized.

Compliance to ethical standards: The study design was approved by the appropriate ethics review boards. All applicable international, national, and institutional guidelines for the care and use of animals were followed

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Conflict of Interest: All the authors here by certify that there is no conflict of interest with any financial issues.

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Figure Captions:

Fig. 1. The optical fluorescence imaging after ICG administrate. Accumulation in the ankle joint is not clear.

Fig. 2. The optical fluorescence imaging after ICG-Lactosome administrate. In the arthritis group, the enhancement of fluorescence luminance of wrist and ankle was observed.

Fig. 3. The optical fluorescence imaging after ICG administrate. Accumulation in the ankle joint is not clear.

Fig. 4. In the simultaneous imaging of 24 hours after ICG-Lactosome administration, it is clear that the fluorescence luminance was rising in arthritis group.

Fig. 5. The fluorescence intensity ratio of each imaging time. Arthritis group was significantly greater than control group in each time.

Fig. 6. The fluorescence intensity ratio in arthritis group was significantly greater at 24 hours after ICG-Lactosome administration.