Lipopolysaccharide (LPS) induces increased epidermal green autofluorescence of mouse

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Abstract

Our recent studies have suggested that characteristic 'Pattern of Autofluorescence (AF)' of each disease could be a novel biomarker for non-invasive diagnosis of acute ischemic stroke, acute myocardial infarction, stable coronary artery disease, Parkinson's disease and lung cancer. While oxidative stress could be a key factor leading to the increased epidermal green AF of the patients' skin, it is necessary to determine if the increased AF may also be produced by other pathological factors of the disorders. Inflammation is a crucial pathological factor in multiple major diseases. In our current study, we used mice exposed to LPS to test our hypothesis that inflammation may also induce increased epidermal green AF. We found that LPS dose-dependently induced significant increases in the epidermal green AF intensity of C57BL/6Slac mouse's ears at both 3 and 7 days after LPS injection. The AF images also exhibited the characteristic structure of the spinous layer. We further found that LPS administration led to keratin 1 degradation of the mouse's ears 7 days after the LPS injection. Collectively, our current study has provided the first evidence suggesting that inflammation could be an important factor that produces the increased epidermal green AF in multiple major diseases. This finding is of critical significance for understanding the characteristic 'Pattern of AF' in the skin of the patients of numerous diseases.

Keywords: Inflammation; LPS; Autofluorescence; Skin; Keratin 1

Introduction

A number of studies have indicated that autofluorescence (AF) of skin or blood can be used for non-invasive diagnosis of diabetes (16) and cancer (24). Our recent study has indicated that UV-induced epidermal green AF could be a novel biomarker for predicting UV-induced skin damage (10). The epidermal green AF appears to be originated from UV-induced keratin 1 degradation (10), which is mediated by UV-induced oxidative stress (12).

Our recent studies have suggested that characteristic 'Pattern of Autofluorescence (AF)' could be a novel biomarker for non-invasive diagnosis of acute ischemic stroke (2), acute myocardial infarction (27), stable coronary artery disease (27), Parkinson's disease (3) and lung cancer (13). While oxidative stress could be a key factor leading to the increased epidermal green AF of the patients' skin, future studies are necessary to determine if the increased AF may also be caused by other pathological factors of the disorders.

Inflammation is a crucial pathological factor in multiple major diseases including cerebral ischemia (20), acute myocardial infarction (18), stable coronary artery disease (11), Parkinson's disease (17) and lung cancer (14). Because inflammation can lead to increased levels of cytokines and oxidative stress (9), we proposed our hypothesis that inflammation may also induce increased epidermal green AF. In our current study, we used mice exposed to lipopolysaccharide **(**LPS) injection to test this hypothesis. Our study has suggested that inflammation could also be an important factor that produces the increased epidermal green AF.

Materials and Methods

All chemicals were purchased from Sigma (St. Louis, MO, USA) except where noted.

Animal Studies

Male C57BL/6Slac mice, ICR mice, and BALB/cASlac-nu nude mice of SPF grade were purchased from SLRC Laboratory (Shanghai, China). All of the animal protocols were approved by the Animal Study Committee of the School of Biomedical Engineering, Shanghai Jiao Tong University.

Administration of LPS

Male C57BL/6Slac mice with the weight of 20 - 25 g were administered with 0.1, 0.5, or 1 mg/kg LPS with intraperitoneal (i.p.) injection. The stock solution of LPS with the final concentration of 0.2 mg/ml was made by dissolving LPS in PBS. The mice were administered with these doses of LPS every 24 hrs.

Imaging of epidermal AF

As described previously (10), a two-photon fluorescence microscope (A1 plus, Nikon Instech Co., Ltd., Tokyo, Japan) was used to image the epidermal AF of the ears of the mice, with the excitation wavelength of 488 nm and the emission wavelength of 500 - 530 nm. The AF was quantified as follows: Sixteen spots with the size of approximately 100 X 100 μm^2 on the scanned images were selected randomly. After

the average AF intensities of each layer were calculated, the sum of the average AF intensities of all layers of each spot was calculated, which is defined as the AF intensity of each spot.

Western blot assays

Western blot assays were conducted as described previously (10): The lysates of the skin were centrifuged at 12,000 *g* for 20 min at 4 ℃. The protein concentrations of the samples were quantified using BCA Protein Assay Kit (Pierce Biotechonology, Rockford, IL, USA). As described previously(22), a total of 50 μg of total protein was electrophoresed through a 10% SDS-polyacrylamide gel, which were then electrotransferred to 0.45 μm nitrocellulose membranes (Millipore, CA, USA). The blots were incubated with a monoclonal Anti-Cytokeratin 1 (ab185628, Abcam, Cambridge, UK) (1:4000 dilution) or actin (1:1000, sc-58673, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) with 0.05% BSA overnight at 4 ℃, then incubated with HRP conjugated Goat Anti-Rabbit IgG (H+L) (1:4000, Jackson ImmunoResearch, PA, USA) or HRP conjugated Goat Anti-mouse IgG (1:2000, HA1006, HuaBio, Zhejiang Province, China). An ECL detection system (Thermo Scientific, Pierce, IL, USA) was used to detect the protein signals. The intensities of the bands were quantified by densitometry using Image J.

Statistical analyses

All data are presented as mean ± SEM. Data were assessed by one-way ANOVA, followed by Student – Newman - Keuls *post hoc* test. *P* values less than 0.05 were considered statistically significant.

Results

The mice were administered with LPS by i.p injection of 0.1, 0.5 and 1 mg/kg LPS. Three days after the LPS administration, the epidermal green AF of their ears was imaged. We found that 0.5 and 1 mg/kg LPS induced significant increases in the skin AF of the ears of C57 mice (Figs. 1A and 1B). The AF images also showed the characteristic structure of the spinous layer (Figs. 1A and 1B), as we observed in the green AF images of UV-exposed ears of the mice (10).

We also determined the epidermal green AF of the ears 7 days after the LPS administration. We found that 0.5 and 1 mg/kg LPS also induced significant increases in the skin AF intensity of the ears (Figs. 2A and 2B). The AF images also showed the characteristic structure of the spinous layer (Figs. 2A and 2B).

Seven days after the mice were administered with 0.1, 0.5 and 1 mg/kg LPS, the keratin 1's level of the ears' skin were determined by Western blot. We found that 1 mg/kg LPS led to a significant decrease in the keratin 1 level of the ears' skin (Figs. 3A and 3B).

Discussion

The major findings of our study include: First, LPS dose-dependently induced significant increases in the epidermal AF intensity of ears' skin of C57BL/6Slac mouse at both 3 and 7 days after LPS injection; second, the AF images show characteristic structure of the spinous layer; and third, LPS administration led to keratin 1 degradation of the mouse's ears 7 days after the LPS injection.

Our recent studies have suggested that characteristic 'Pattern of Autofluorescence (AF)' could be a novel biomarker for non-invasive diagnosis of acute ischemic stroke (2), myocardial infarction (27), stable coronary artery disease (27), Parkinson's disease (3) and lung cancer (13). While oxidative stress could be a key factor leading to the increased epidermal green AF of the patients' skin (12), our current study has suggested that inflammation could also be an important factor that produces the increased AF intensity: Our study has shown that inflammation can induce significant increases in the epidermal AF intensity, which has similar structure as that observed in the ears exposed to UV-induced oxidative stress (10). Because inflammation is a crucial pathological factor in multiple major diseases, the inflammation may also contribute to the increased epidermal AF of the patients, which may become a novel biomarker for non-invasive and efficient diagnosis of multiple major diseases. Since the mechanisms underlying the increased generation of AF intensity are of crucial importance for establishing the novel diagnostic technology - the 'Pattern of AF' technology, the findings of our current study are critical for establishing the diagnostic technology.

Keratins play multiple significant roles in epithelium, including intermediate filament formation (21), inflammatory responses (5,19) and cellular signaling (1,6).

Keratin 1 and its heterodimer partner keratin 10 are the major keratins in the suprabasal keratinocytes of epidemis (4,15,23), which is a hallmarker for keratinocyte differentiation (26). As found in UV-exposed mouse skin (12), our current study has also found that LPS induced keratin 1 degradation of the mouse's ears 7 days after the LPS injection. Therefore, similar with the mechanisms underlying the UV-induced epidermal green AF, inflammation may also induce increased epidermal green AF by producing keratin 1 degradation.

Since inflammation is a key common pathological factor in numerous diseases such as Alzheimer's disease (7), multiple sclerosis (8) and amyotrophic lateral sclerosis (25), our study has suggested that the inflammation in these diseases may also induce significant increases in epidermal green AF. Therefore, these diseases may also be diagnosed on the basis of the inflammation-induced AF.

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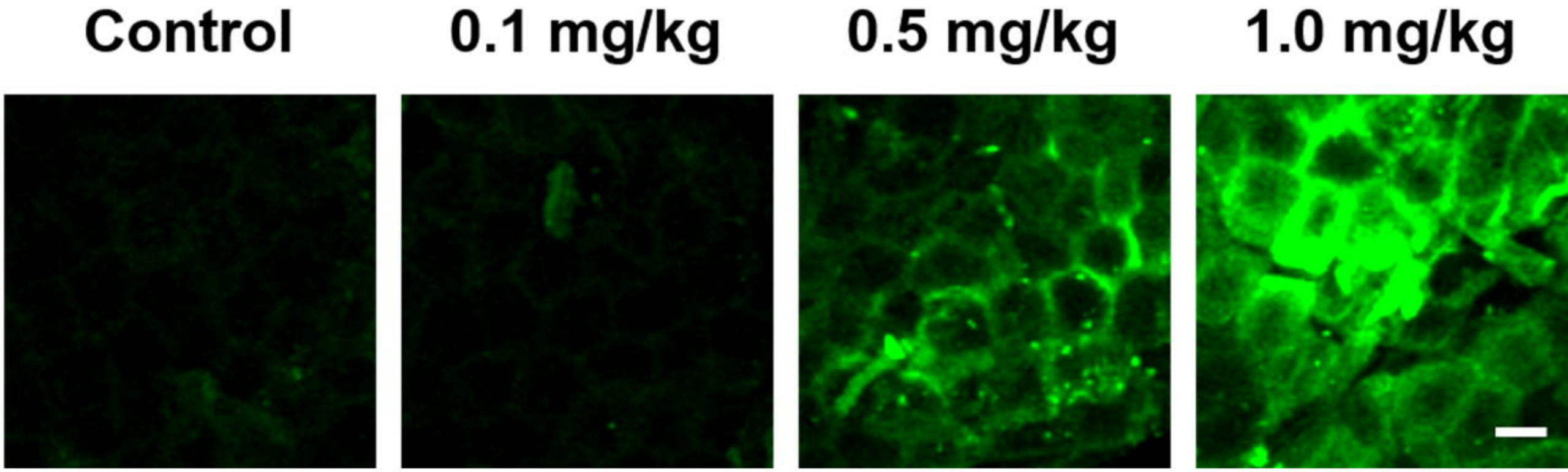
Figure Legends:

Figure 1. LPS dose-dependently induced increases in the epidermal green AF of C57 mouse's ears 3 days after LPS injection. (A) i.p injection of 0.1, 0.5 and 1 mg/kg LPS does-dependently increased the skin AF of the ears, assessed at 3 days after the LPS injection. Excitation wavelength = 488 nm and emission wavelength = 500 - 530 nm. Scale bar = 20 μm. (B) Quantifications of the AF showed that 0.5 and 1 mg/kg LPS induced significant increases in the skin AF of the ears. Three days after mice were administered with 0.1, 0.5 and 1 mg/kg LPS, the epidermal green AF images of the mice' ears were taken under a two-photon fluorescence microscope. N = 6. **, $P < 0.01$; ***, $P < 0.001$.

Figure 2. LPS dose-dependently induced increases in the epidermal green AF of C57 mouse's ears 7 days after LPS injection. (A) i.p injection of 0.1, 0.5 and 1 mg/kg LPS does-dependently increased the skin AF of the ears, assessed at 7 days after the LPS injection. Excitation wavelength = 488 nm and emission wavelength = 500 - 530 nm. Scale bar = 20 μm. (B) Quantifications of the AF showed that 0.5 and 1 mg/kg LPS induced significant increases in the skin AF of the ears. Seven days after mice were administered with 0.1, 0.5 and 1 mg/kg LPS, the epidermal green AF images of the mice' ears were taken under a two-photon fluorescence microscope. N = 6. ##, $P < 0.01$; ***, $P < 0.001$.

Figure 3. LPS administration led to keratin 1 degradation of C57 mouse's ears. (A) Western blot assays showed that i.p injection of 1 mg/kg LPS led to a decrease in the keratin 1 level of the ears, assessed at 7 days after the LPS administration. (B)

Quantifications of the Western blot showed that 1 mg/kg LPS led to a significant decrease in the keratin 1 level of the ears. Seven days after mice were administered with 0.1, 0.5 and 1 mg/kg LPS, the keratin 1's level of the ears were determined by Western blot . *, $P < 0.05$. N = 12.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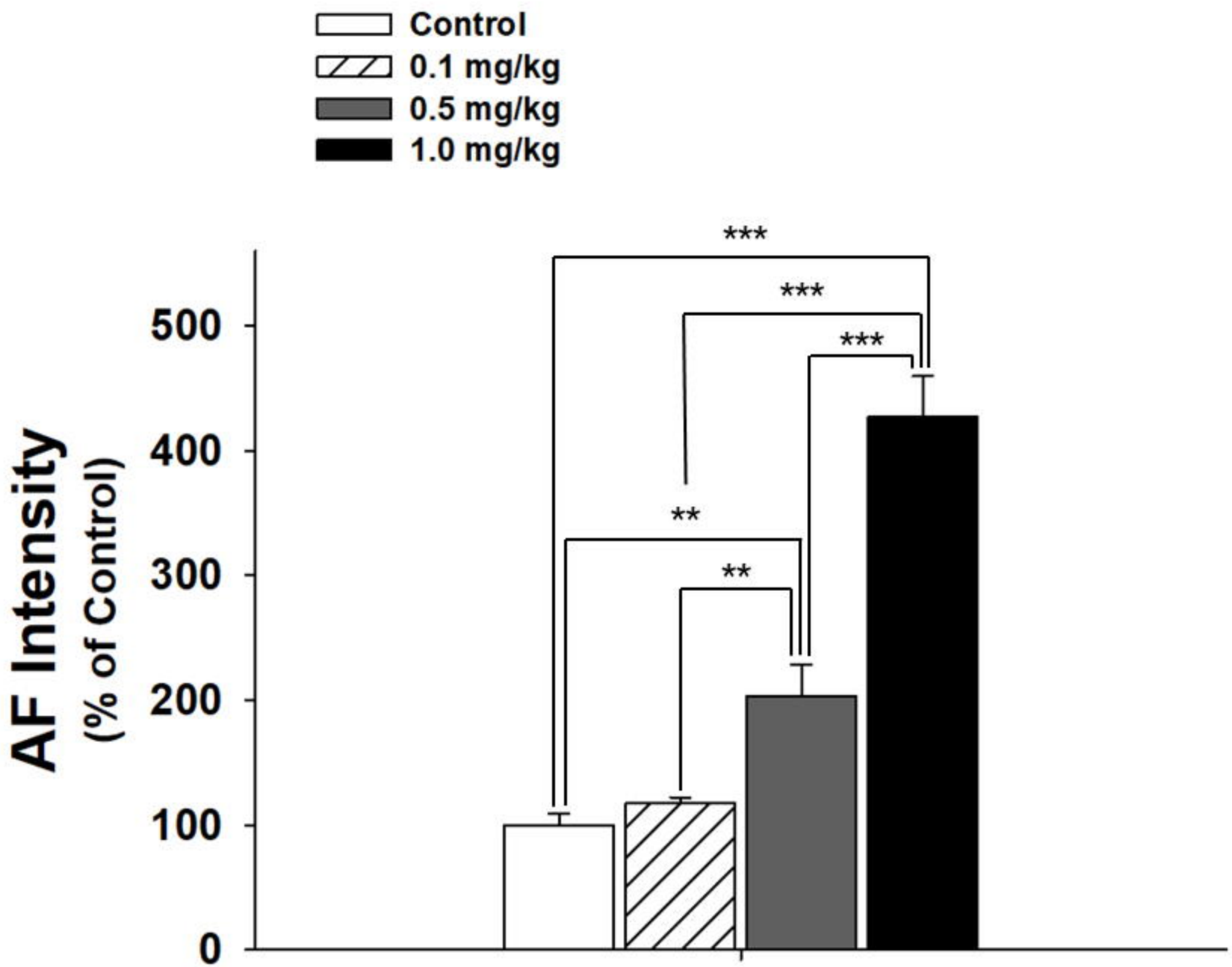
1.0 mg/kg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AF Intensity
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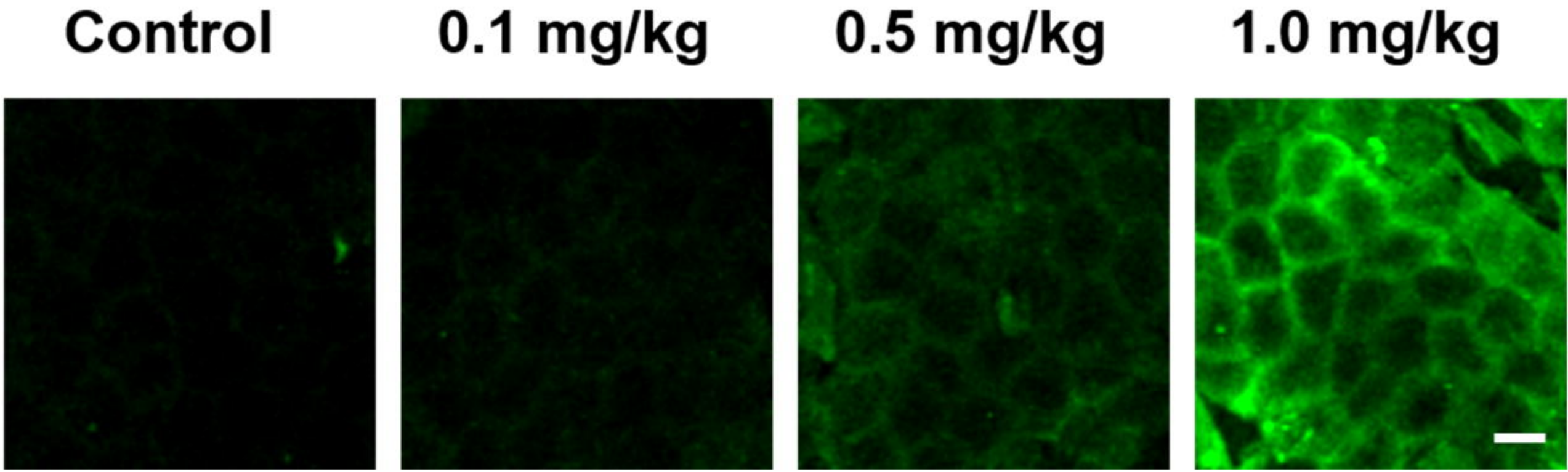
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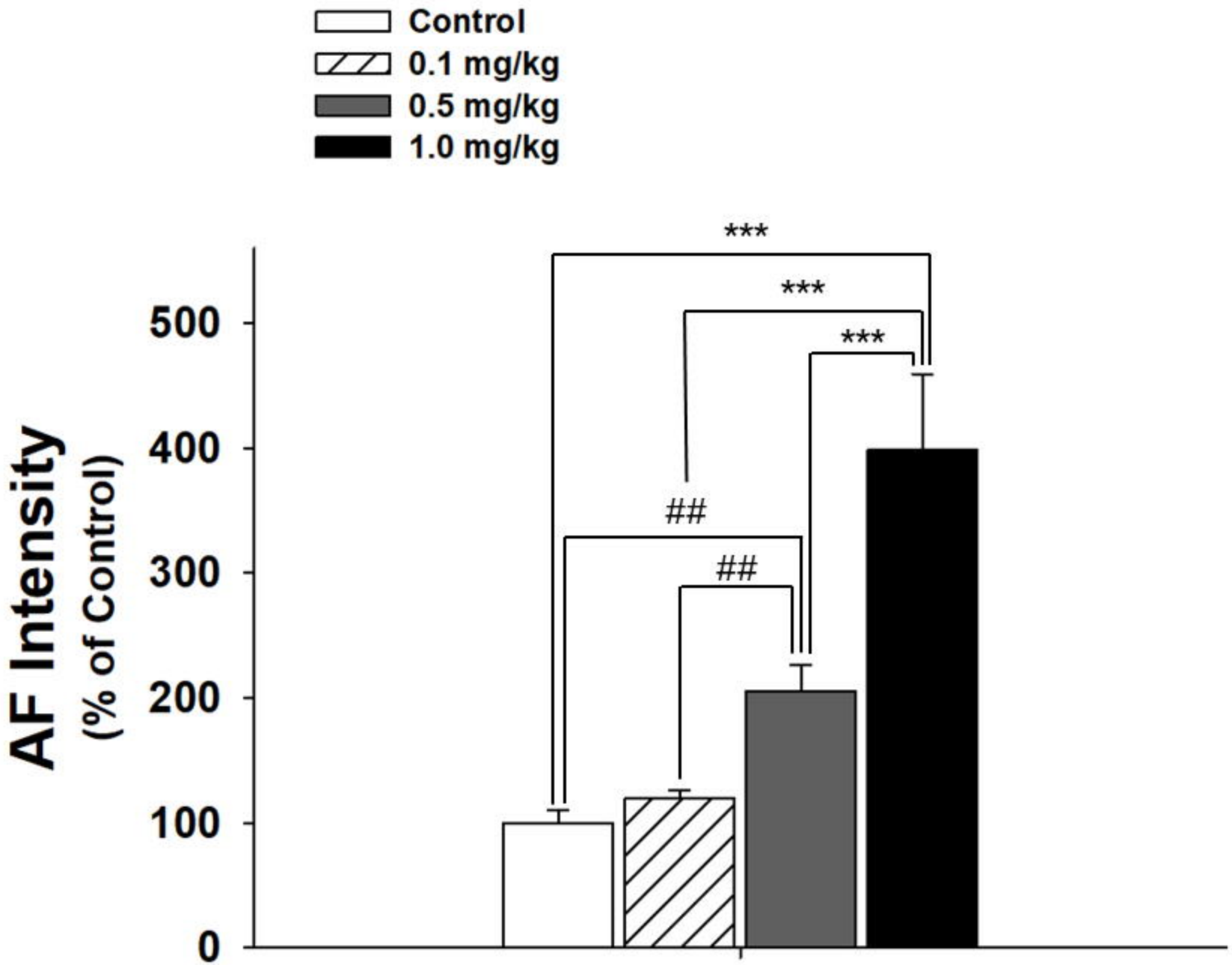
Control
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(% of Control)
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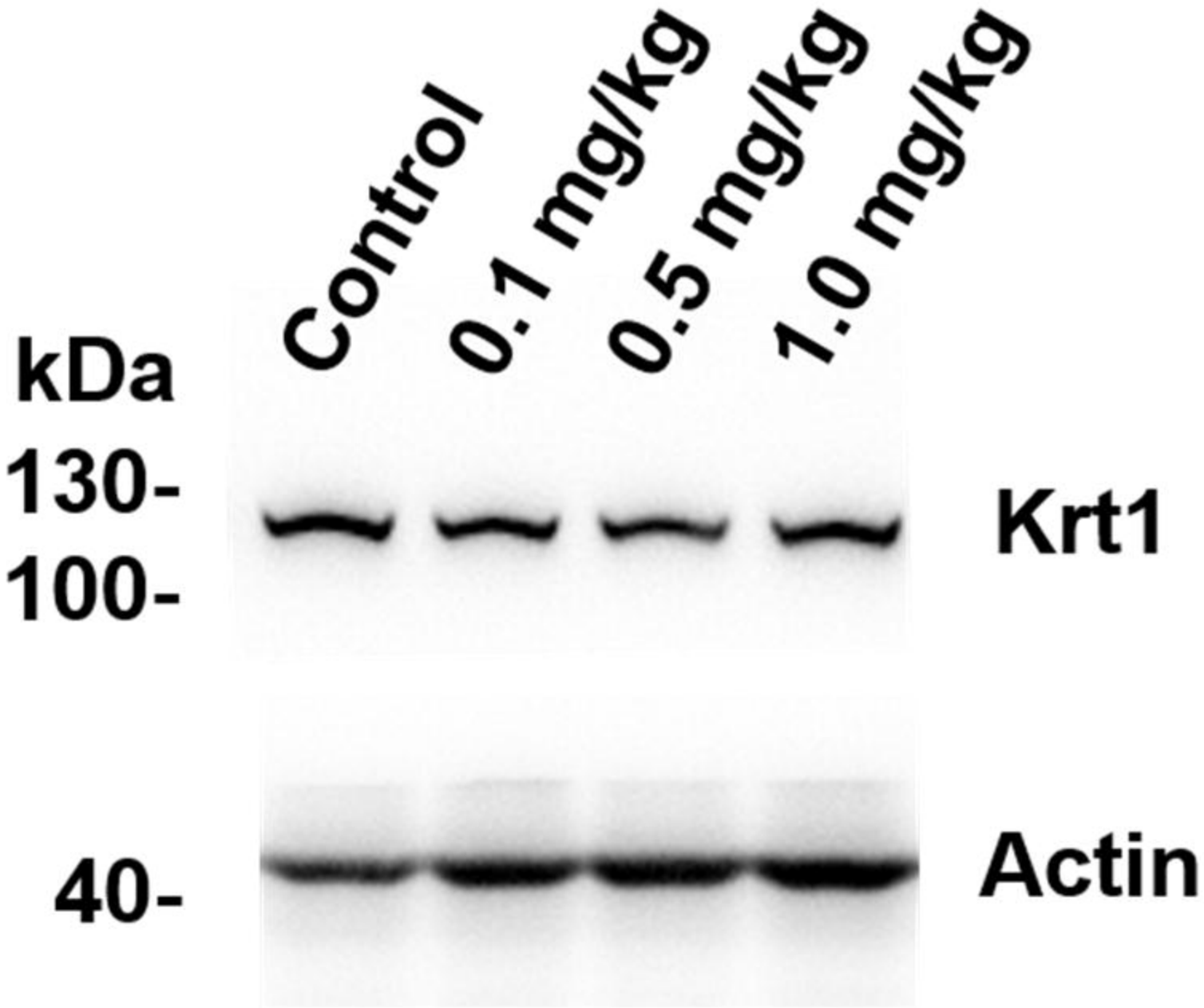
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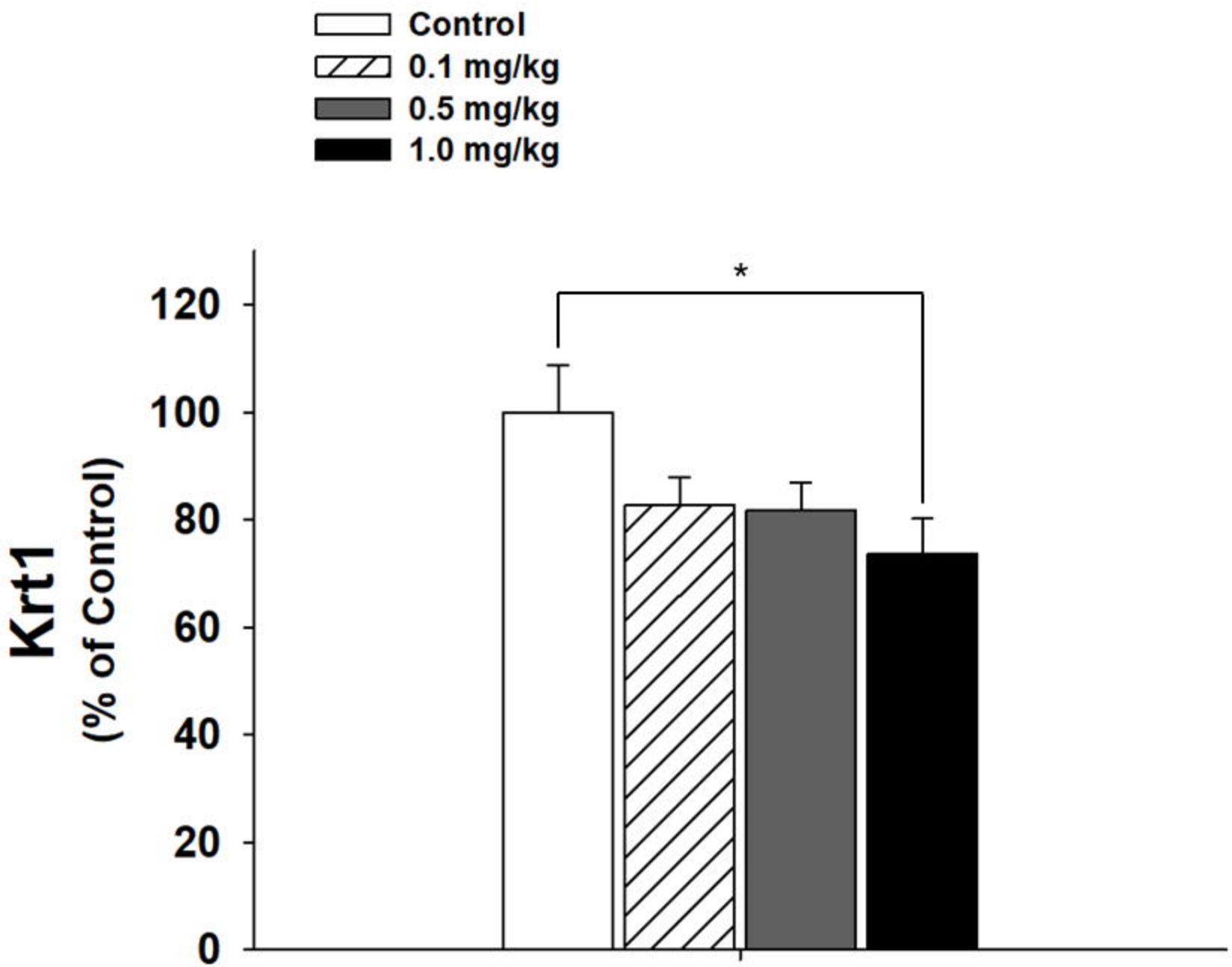
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Krt1
(% of Control)