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Emamectin Benzoate and Microplastics Led to Skeletal Muscle Atrophy in Common Carp via Induced Oxidative Stress, Mitochondrial Dysfunction, and Protein Synthesis and Degradation Imbalance

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ABSTRACT: Pesticides and plastics have brought convenience to agricultural production and daily life, but they have also led to environmental pollution through residual chemicals. Emamectin benzoate (EMB) is among the most widely used insecticides, which can cause environmental pollution and harm the health of organisms. Additionally, microplastics (MPs), a relatively new type of pollutant, not only are increasing in residual amounts within water bodies and aquatic organisms but also exacerbate pollution by adsorbing other pollutants, leading to a mixed pollution scenario. Nevertheless, the toxicity and mechanism of EMB and MPs on common carp skeletal muscle have not been elucidated. Therefore, we established exposure models for EMB and MPs, and methods such as hematoxylin and eosin staining, immunofluorescence staining, JC-1 staining, and western blotting were employed to investigate the underlying mechanisms of skeletal muscle damage. The results of in vivo and in vitro experiments indicated that exposure to EMB or MPs led to oxidative stress, which in turn caused mitochondrial fusion/fission imbalance (with decreased Mfn1, Mfn2, and OPA1 and increased DRP1), reduced mitochondrial membrane potential, decreased ATP content, reduced protein synthesis, and increased degradation, ultimately resulting in skeletal muscle atrophy. Joint exposure caused more severe damage than single exposure, and the addition of NAC can effectively alleviate skeletal muscle atrophy. In summary, exposure to EMB and/or MPs induced excessive reactive oxygen species (ROS) production, giving rise to mitochondrial dysfunction and an imbalance in skeletal muscle protein synthesis and degradation, ultimately resulting in skeletal muscle atrophy in common carp.

KEYWORDS: *EMB, MPs, oxidative stress, protein synthesis and degradation, skeletal muscle atrophy*

1. INTRODUCTION

Emamectin benzoate (EMB) is a newly developed and extremely effective semisynthetic antibiotic insecticide synthesized from fermented product Abamectin B1; it is widely used as a substitute for highly toxic pesticides due to its highly efficient and low residue biopesticides and is generally applied for controlling pests in vegetables, fruit trees, cotton, and field crops. Furthermore, EMB is frequently added to fish feed to combat parasitic infestations in fish farming. The long degradation half-life and hydrophobicity of EMB mean that it can persist in fish farms and marine sediments, leading to a major exposure threat to benthic organisms.[1] In the aquatic environment, whether or not EMB is used as an insecticide or a parasite preventive drug, EMB will continue to accumulate in fish or organisms. EMB, due to its high lipophilicity, is highly prone to penetrating the cell membrane and enter the cytoplasm, posing potential safety risks.[2] A study has shown that prolonged exposure to pesticides can cause harmful influences on the cells of nontarget organisms.[3] EMB enhanced oxidative stress induced developmental toxicity and cardiac toxicity in zebrafish, reducing hatching rate.[4] In addition, EMB exhibits neurotoxicity, immunotoxicity, and reproductive toxicity toward rats and aquatic animals.[5−7] EMB disrupts the hypothalamic-pituitary-ovarian (HPO) axis through oxidative stress, causing a disruption in hormone levels in female rats.[8] Exposure to 200 μM EMB disrupts spindle organization, chromosome alignment, and microfilament polymerization, perturbs mitochondrial distribution, and induces a raise in the production of reactive oxygen species (ROS), ultimately resulting in DNA damage and early cell apoptosis.[9] After feeding Nile tilapia with EMB at a dose of 50 μg/kg/day for 14 days, significant changes were observed in its blood parameters.[10] While numerous investigations have established EMB's toxicity in a range of species, nevertheless, there is limited understanding regarding the mechanism by which EMB exerts its toxicity to common carp skeletal muscle.

Because of the large-scale production and poor management of plastic products, plastic pollution has rapidly increased and attracted widespread global attention.[11,12] Over time, plastic pollutants gradually degrade into fragments with a diameter less than 5 mm, known as microplastics (MPs).[13] MPs enter

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environments such as soil, rivers, lakes, and oceans, causing immeasurable negative impacts on ecosystems and biodiversity and endangering the health of organisms through the accumulation of food chains.[14−16] In marine and freshwater ecosystems, the concentration of MPs rapidly increases, even reaching 4500 μg/L.[17] Plastic debris in the environment may have an impact on aquatic organisms such as fish, seabirds, or sea lions through entanglement or ingestion.[18] Recently, the detection of MPs has been confirmed in fish, sea creatures, and mammals.[19−21] More and more evidence suggested that exposure to MPs has neurotoxicity, immunotoxicity, and reproductive toxicity.[22,23] In addition, muscles have also been shown to be the target tissue of MPs particles, which can penetrate the epidermis and enter muscle tissue, thereby inhibiting the growth of goldfish fry.[24] Exposure to MPs can also exacerbate oxidative stress in skeletal muscles.[25] It is worth noting that in natural environments, aquatic organisms often come into contact with pesticides and MPs together.[26] The coexposure of pesticides and MPs causes liver necrosis and congestion as well as gills edema and lamellar fusion in piaractus mesopotamicus juvenile fish.[27]

Oxidative stress is triggered by a lack of balance between the body's production of oxidants and antioxidants, and exposure to environmental pollutants enhances the accumulation of ROS, causing oxidative stress. Mitochondria are the main site for generating ROS, and ROS overproduction can result in mitochondrial dysfunction, structural impairments, and disruptions in energy metabolism. Multiple studies have found that environmental pollutants such as pesticides and microplastics cause oxidative stress, and mitochondrial damage, and dysfunction.[28−30] Copper or arsenic exposure resulted in oxidative stress and mitochondrial dysfunction, which in turn led to skeletal muscle damage in chickens.[31] The appearance of oxidative stress and mitochondrial dysfunction also affects the balance between protein anabolism and catabolism. Mitochondrial dysfunction decreases protein synthesis and enhances protein degradation.[32] Research has found that resveratrol (RSV) improves protein metabolism to prevent muscle atrophy by restoring mitochondrial function and combating oxidative stress.[33] The ubiquitin proteasome system (UPS) and the autophagy lysosome system (ALS) are the two major intracellular protein degradation pathways that are tightly controlled within the cell and fulfill diverse physiological and pathological functions.[34] The common carp is a popular freshwater fish consumed extensively in Asia, with characteristics such as easy aquaculture, low-cost, and rich nutrition, accounting for one-tenth of the global freshwater aquaculture industry.[35] Among them, muscle is the organ with the largest proportion in common carp, which contains abundant protein and amino acids and is a significant source of protein in the daily diet. It is concerning that various environmental pollutants have been detected in aquatic systems, such as pesticides, plastics, heavy metals, flame retardants, etc.[36−38] Due to high-density aquaculture and the persistent presence of various environmental pollutants, the normal physiological state of muscles is disrupted, leading to the deterioration of meat quality, and the specific mechanism behind it is still unclear. Therefore, it is imperative that we must focus on the direct toxicity of pollutants to aquatic organisms and the potential threat to human health posed by aquatic products rich in harmful substances. Based on this, this study constructed an EMB and/or MPs exposure model for common carp skeletal muscle and skeletal muscle myoblasts cell lines (CCM cells); hematoxylin and eosin (H&E) staining, JC-1 staining, immunofluorescence (IF) staining, western blotting, and other experimental methods were applied to detect the influences of EMB or MPs exposure on common carp skeletal muscle and to determine the potential regulatory mechanism. Compared to studying the impact of individual pollutants, studying the synergistic effects of various pollutants provides more comprehensive guidance for protecting aquatic environments. This method can furnish a broader perspective on the necessities for safeguarding the environment in actual scenarios.

2. MATERIALS AND METHODS

2.1. Establishment and Grouping of EMB and/or MPs Exposure Models. All experimental protocols were in strict compliance with the moral guidelines of the Ethics Committee at Northeast Agricultural University and the EU's directive on laboratory animal protection (SM-11). A total of 60 healthy common carp (Harbin Changling Lake Fish Farm, 206.34 ± 8.63 g) were randomly distributed into buckets filled with 15 L of water, with each group consisting of 3 barrels, and 5 common carp per barrel. Following a 1-week acclimation period, the common carp were evenly assigned to four experimental groups: a control group (C), an emamectin benzoate (EMB) exposure group, a microplastic (MPs) exposure group, and a combined emamectin benzoate and microplastic (EMB + MPs) exposure group. The concentrations of EMB (E396669, purity >95%, Aladdin) and MPs (PS-MPs, 10 μm, Fengtai polymer material) were 2.4 and 500 μg/L, respectively.[39,40] The fish were provided with feed twice each day, and a half-body water exchange method was used to change the water (dechlorinated tap water), ensuring the concentrations of EMB and MPs were in water. There were no mortalities among the common carp throughout the entire exposure phase. The exposure test period was 31 days, the common carp were painlessly terminated, and their skeletal muscle tissues were rapidly collected, with samples being preserved in liquid nitrogen and a 4% paraformaldehyde solution (Macklin, Shanghai, China).[41]

2.2. H&E Staining. Skeletal muscle fixed in 4% paraformaldehyde was subjected to fixation, sectioning, and staining experiments. Optical microscopy was utilized to observe pathological alterations in skeletal muscle, and the resulting images were gathered for analysis.[42] H&E staining was applied to observe skeletal muscle, and ImageJ software was applied for quantitative analysis of the obtained results to calculate the average diameter and average cross-sectional area (CSA) of skeletal muscle fibers.

2.3. IF Staining. Skeletal muscle tissue fixed in 10% paraformaldehyde was embedded in paraffin and dehydrated using gradient alcohol dewaxing. Subsequently, EDTA antigen repair buffer (pH 8.0) was used to perform antigen repair on tissue sections in a microwave oven, sealed with serum (5% FBS TBST) for 30 min, and incubated overnight with primary antibody (LC3B, ABclonal,1:500; BNIP3, ABclonal, 1:200; MURF1, ABclonal,1:200; MAFbx, ABclonal,1:200). Next, the slices were washed with phosphate-buffered saline (PBS), incubated with secondary antibodies (Dylight 488 and 594 goat antirabbit IgG, Biodragon, China), and allowed to stand for 30 min. After washing with PBS after sealing, a fluorescence microscope was employed to observe and capture images. We quantified the fluorescence intensity of the IF staining results using the ImageJ system.

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Table 1. Gene-Specific Primer for qRT-PCR

gene	forward primer (5′-3′)	reverse primer (5′-3′)
DRP1	TCCCGAGAGCCAAGAAGAAATACG	TGTAACTGAGGCACCGCAAGAG
Mfn1	AGTGTATCTCGCAGTCAGCAGTG	CTTCTCCGCCGCTTCAATGTTG
Mfn2	TCGTCACCGCCAAGAAGAAGATC	ACCTCTGCCACCTGCTCCTC
MyoD	TCTTACTTCAACGACACGCCAAATG	GGACTGACAGCACGGGACAC
MyoG	CTTGGTCTGTCCTTGTCTCCTCAC	TTCAACCGTCTCTTCTCCCTCAAAG
Myf5	CCCAGTCAGCGTCTTCCCAAG	GGCAGGCTGTAGTAGTTCTCCAC
Myh1	AAGCAGAAGCAGCGAGAGGAAC	TTGACTCTTGGATGGCACAGACC
Myh2	GCTTACGGTAATGCCAAGACAGTG	CTTCTCCAGCAGGTAGGTCTCAATG
OPA1	GGATGAGGAAGAGTGTGAAGGAAGG	GGCAAGTCAACGAGCACCATTC
β-actin	GATGGACTCTGGTGATGGTGTGAC	TTTCTCTTTCGGCTGTGGTGGTG

2.4. Cultivation and Vitality Determination of CCM Cells. The CCM cells (Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences) were cultured in L15 (Thermo Fisher Scientific, USA) medium containing 10% FBS (VivaCell, China), 1% penicillin streptomycin amphotericin B solution (Beyotime, China), 1% L-glutamine (Gibco), and 0.5% common carp serum (Hongquan Biotechnology Co., Ltd., China) and cultivated them at 28 °C in a CO_2 free incubator. When the CCM cells' density reached 95%, the CCM cells were passaged.

The solid form of EMB was first dissolved in DMSO to create a stock solution at a concentration of 1 mM, which was then further diluted with growth medium to achieve final concentrations of 0, 0.1, 0.5, 1, 2, 4, 6, 8, and 10 μM. Similarly, the solid form of MPs was dissolved in PBS to create a stock solution at a concentration of 10 mg/mL, which was then diluted to 0, 1, 10, 50, 100, 200, 400, 500, 600, 800, and 1000 μg/mL. NAC (NAC, A7250, Sigma) was dissolved in DMSO to make a stock solution of 1 M, which was then diluted to a final working concentration of 1 mM. The CCM cells were plated in a 96-well plate and exposed to varying dose intensity of EMB (0, 0.1, 0.5, 1, 2, 4, 6, 8, and 10 μM) and/or MPs (0, 1, 10, 50, 100, 200, 400, 500, 600, 800, and 1000 μg/mL) for 24 h when the cells reach a stable adherent state. Afterward, the culture medium was exchanged for a solution containing 10% CCK-8 reagent (40203ES80, Yeasen) for assay purposes and further cultured at 28 °C for 2 h. Subsequently, the absorbance at 450 nm was detected using a Multiskan SkyHigh (Thermo).

2.5. Oxidative Damage Index and ATP Content Detection. A 0.1 g portion of skeletal muscle was weighed from each group, and a 10% homogenate was prepared using physiological saline. The mixture was centrifuged at 7500 rpm (10 min, 4 °C), the supernatant was collected and operated according to the reagent kit (Nanjing Jiancheng, China) to detect oxidative stress indicators and ATP content (SOD A001−3, GSH A006−1−1, CAT A007−1−1, MDA A003−1, T-AOC A015−2−1, A095−1−1 ATP), the OD value was measured, and calculations were performed.

2.6. ROS Staining. Each group of CCM cells (1×10^6 cells/mL) was collected in 15 mL centrifuge tubes, spun at 1000 rpm for 5 min, after which the supernatant was removed and the cells were rinsed twice using PBS. A diluted DCFH-DA probe (PBS:DCFH-DA = 1000:1, freshly prepared and used) was added for 30 min, the cells were subjected to another two washes using PBS and a new PBS was added, and fluorescence microscopy (Olympus, IX53, Japan) was employed to observe ROS levels.

2.7. JC-1 Staining. The mitochondrial membrane potential (MMP) in each group was assessed using a JC-1 fluorescent probe assay kit from Beyotime, China. Under high MMP conditions, JC-1 forms polymers in the mitochondrial matrix, emitting red fluorescence, whereas low MMP leads to JC-1 to not aggregate in the matrix of mitochondria but instead to become a monomer, which emit green fluorescence. The CCM cells, in 6-well plates, were exposed to varying levels of EMB and/or MPs dosages for 24 h. Subsequently, the cells were stained using JC-1 staining solution for 30 min, after which fluorescence microscopy (Olympus, IX53, Japan) was employed to view the images.

2.8. RNA Isolation and Real-Time Quantitative PCR (qRT-PCR) Analysis. Tge Trizol reagent method was used for total RNA extraction.[43] The reverse transcription kit (MR05101M, Monad) was used for RNA reverse transcription, and the obtained cDNA was stored at −20 °C. SYBR Green fluorescent dye (AQ132−11, Transgen) was used for quantitative detection in the LineGene9600Plus detection system, and the total reaction system was 10 μL (0.3 μL particular forward primer, 0.3 μL particular reverse primer, 5 μL SYBR Green fluorescent dye, 1 μL of diluted cDNA, 3.4 μL of double distilled water). β-actin was adopted as a control protein for consistency checks. The $2^{-\Delta\Delta CT}$ technique was employed for quantifying the levels of the target gene. Table 1 features the primer sequences.

2.9. Western Blot Analysis. Protein expression was analyzed using the western blot technique.[43] Freshly prepared tissue lysate (PMSF+IP = 100:1, Beyotime, China) was used to extract total protein, and SDS-PAGE electrophoresis and membrane transfer were then performed. 5% skimmed milk powder was sealed for 90 min and incubated overnight with primary antibody; the next day, the horseradish-peroxidase labeled secondary antibody was incubated, and the Super ECL Kit (MA0186, Meilunbio) gel imaging system was used for image acquisition. β-actin was chosen as a reference control. We quantified the optical density values of protein bands by using the ImageJ system. All antibody sources and dilution rates are shown in Table 2.

2.10. Data Statistics and Analysis. GraphPad Prism 9.0 software was employed for data processing and statistical analysis. One-way ANOVA and the Tukey method were employed to assess the significance of variances among various groups. All data were presented as mean ± standard deviation. The number of repetitions of the experiment was $n = 3$. $P < 0.05$ indicated statistically significant differences, identified by distinct superscript letters. $P \geq 0.05$ indicated nonsignificant differences, represented by the same superscript letter.

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Table 2. Antibody Information

antibodies	dilution ratio	manufacturer
Mfn1	1:2000	ABclonal, Wuhan, China
Mfn2	1:2000	ABclonal, Wuhan, China
OPA1	1:2000	ABclonal, Wuhan, China
DRP1	1:2000	ABclonal, Wuhan, China
LC3B	1:1000	ABclonal, Wuhan, China
BNIP3	1:1000	ABclonal, Wuhan, China
MAFbx	1:1000	ABclonal, Wuhan, China
MURF1	1:1000	ABclonal, Wuhan, China
MyoD	1:1000	ABclonal, Wuhan, China
MyoG	1:1000	ABclonal, Wuhan, China
p-AKT	1:500	Wanlei, Shenyang, China
IGF-1	1:500	Wanlei, Shenyang, China
p-p70S6K	1:500	Wanlei, Shenyang, China
β-actin	1:10000	Bioss, Beijing, China

3. RESULT ANALYSIS

3.1. The Effect of EMB and/or MPs Exposure on Common Carp Skeletal Muscle. H&E staining was conducted to explore the influences of EMB or MPs on the regeneration of skeletal muscle in common carp. As shown in Figure 1A–C, exposure to EMB and MPs resulted in muscle atrophy. The average diameter of muscle fibers in the C group was 114.19 μm, surpassing the EMB group by 22.91%, the MPs group by 43.30%, and the EMB + MPs group by 53.18%. Additionally, in the C group, the CSA of the muscle fibers in the C group was 11767.40 μm^2, surpassing the EMB group by 45.98%, the MPs group by 70.94%, and the EMB + MPs group by 79.93%.

Moreover, at the mRNA expression level, exposure to EMB and/or MPs significantly inhibited the expression of myogenic differentiation markers (MyoD, MyoG, Myh1, Myh2, and Myf5) (Figure 1D). At the same time, similar results were obtained at the protein levels (Figure 1E,F), and MyoD and MyoG were inhibited by exposure to EMB and/or MPs. These results indicated that exposure to EMB and/or MPs leaded to a reduction in skeletal muscle regeneration efficiency, ultimately resulting in skeletal muscle atrophy.

3.2. The Effect of EMB and/or MPs Exposure on Differentiation of CCM Cells. In order to further inspect the influence of EMB or MPs on skeletal muscle regeneration, in vitro experiments were conducted using CCM cells as the research subjects. Initially, the impact of EMB or MPs on CCM cell survival was assessed via the CCK-8 assay method. The vitality of CCM cells was notably reduced with increasing doses of EMB or MPs, exhibiting concentration dependence

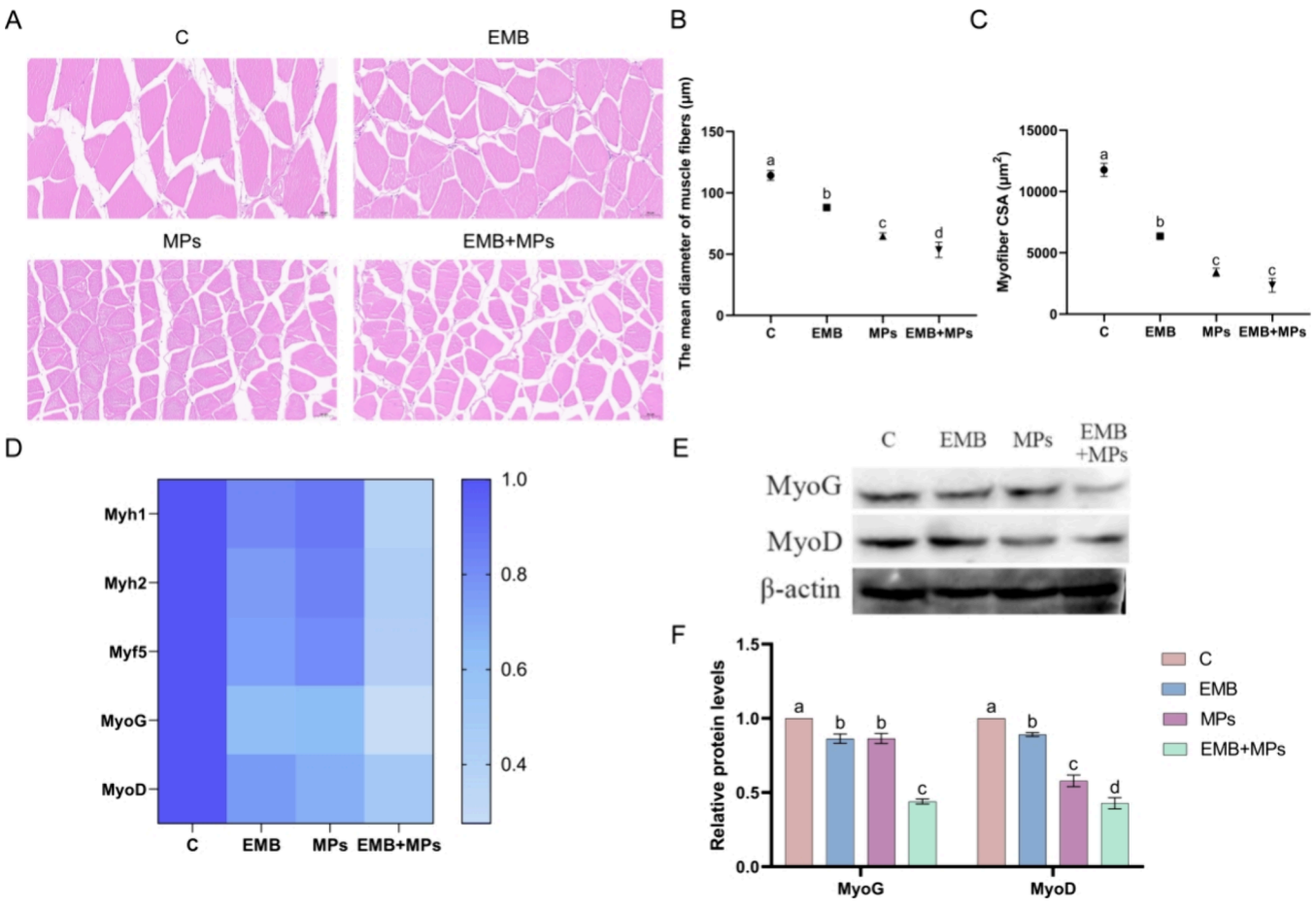

Figure 1. Exposure to EMB and/or MPs led to skeletal muscle atrophy in common carp. (A) H&E staining results of common carp skeletal muscle (20×), bar = 50 μm. (B) The average diameter of muscle fibers. (C) The average cross-sectional area (CSA) of muscle fibers. (D) The mRNA expression results of markers related to myogenic differentiation. (E, F) Protein levels and quantitative analysis results of markers related to myogenic differentiation. The same superscripts indicated that the difference was not statistically significant ($P \geq 0.05$), while different superscripts indicated that the difference was statistically significant ($P < 0.05$), the same applies below.

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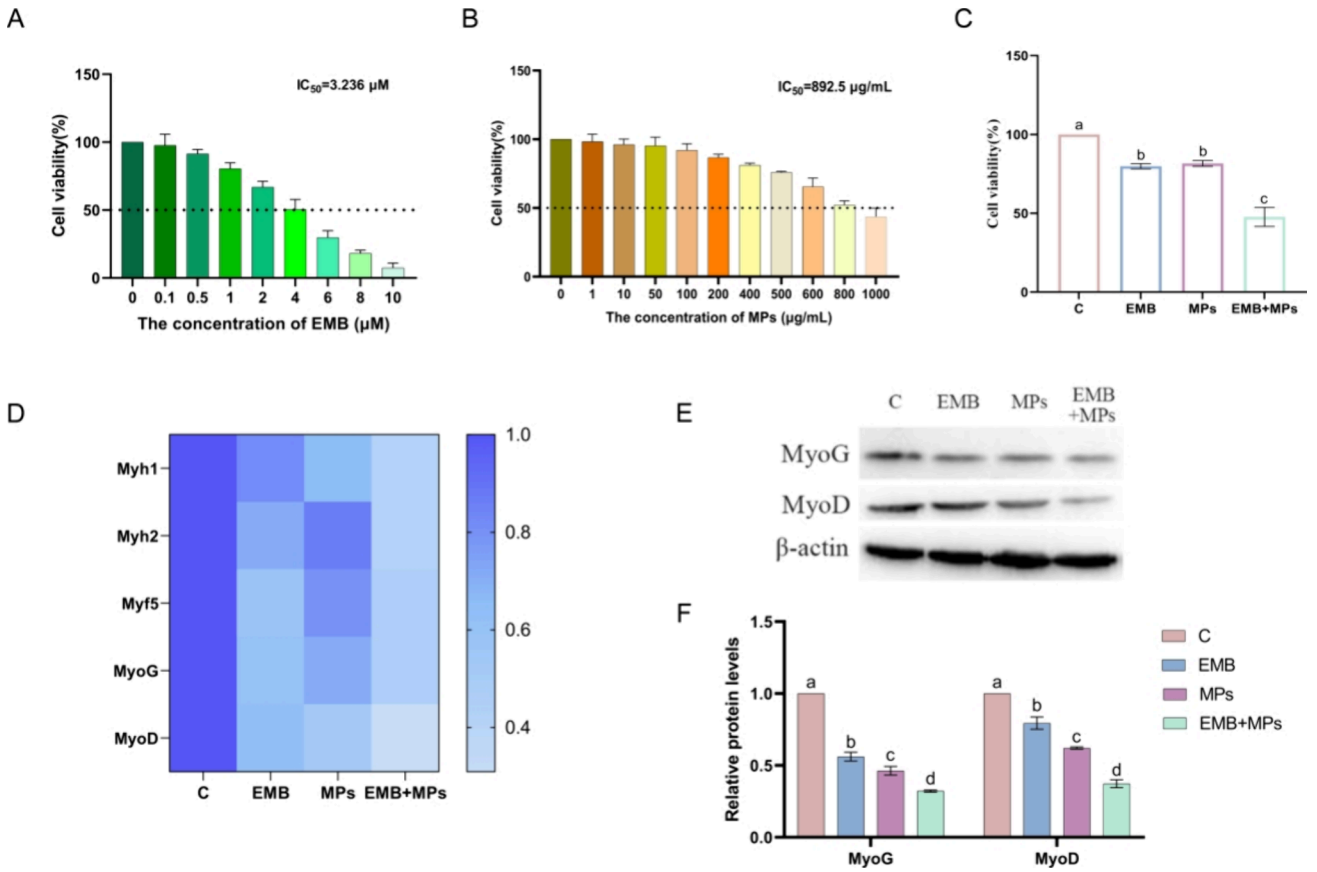

Figure 2. Exposure to EMB and/or MPs inhibited the differentiation of CCM cells. (A−C) The viability of CCM cells treated with EMB and/or MPs for 24 h. (D) The mRNA expression results of markers related to myogenic differentiation. (E, F) Protein levels and quantitative analysis results of markers related to myogenic differentiation.

(Figure 2A,B). When EMB and MPs were coexposed at concentrations of 1 μM and 400 μg/mL, respectively, the viability of cells was significantly lower than that of either treatment alone (Figure 2C). Those findings suggested that EMB and/or MPs reduced the viability of CCM cells.

In addition, at the mRNA expression level, exposure to EMB and/or MPs significantly inhibited the expression of myogenic differentiation markers (MyoD, MyoG, Myh1, Myh2, and Myf5) and the protein levels (Figure 2D−F). These results indicated that exposure to EMB and/or MPs inhibited the myogenic differentiation of CCM cells.

3.3. The Effect of EMB and/or MPs Exposure on Oxidative Stress and ROS Production. Oxidative stress is generally considered the initiator of numerous diseases or pathological damage. This experiment first tested the antioxidant levels of skeletal muscle exposed to EMB and/or MPs. As shown in the figure (Figure 3A−E), EMB and/or MPs treatment significantly inhibited the activities of antioxidant enzymes (SOD and CAT) and T-AOC, reduced the level of GSH, and increased the level of MDA. The oxidative stress caused by the coexposure of EMB and MPs was more pronounced.

Next, in cell experiments, the ROS levels of CCM cells treated with EMB and MPs were detected. EMB and/or MPs treatment significantly increased the generation of ROS in CCM cells, and the coexposure of EMB and MPs produced significantly higher levels of ROS than exposure to EMB or MPs alone (Figure 3F,G). NAC treatment notably augmented the protein levels of MyoD and MyoG in CCM cells exposed to EMB and MPs (Figure 3H,I). These results indicated that exposure to EMB or MPs caused oxidative stress and induced excessive production of ROS, and their coexposure exacerbated oxidative stress levels. Additionally, inhibiting the excessive production of ROS restored the expression of the differentiation markers in CCM cells.

3.4. The Effect of EMB and/or MPs Exposure on Mitochondrial Function. In order to further evaluate whether muscle damage caused by exposure to EMB or MPs was related to mitochondrial dysfunction, this experiment detected changes in mitochondrial dynamics, mitochondrial membrane potential, and ATP content. It was found that exposure to EMB and/or MPs resulted in mitochondrial motility imbalance, with decreased mRNA expression and protein levels of mitochondrial fusion proteins (Mfn1, Mfn2, and OPA1) and increased mRNA expression and protein levels of mitochondrial fission protein (DRP1) (Figure 4A−F). After treatment with EMB or MPs at their respective concentrations, there was a reduction in the polymerization of JC-1 aggregates and an increase in JC-1 monomers, indicating that this exposure led to a decline in MMP (Figure 4G). In addition, exposure to EMB and MPs also resulted in a marked lowering of the ATP content (Figure 4H). More interestingly, the coexposure of EMB and MPs exacerbated mitochondrial dysfunction in skeletal muscle and CCM cells. These results indicated that muscle atrophy caused by exposure to EMB and MPs was accompanied by mitochondrial dysfunction.

3.5. The Effect of EMB and/or MPs Exposure on Protein Synthesis and Degradation Balance. The result

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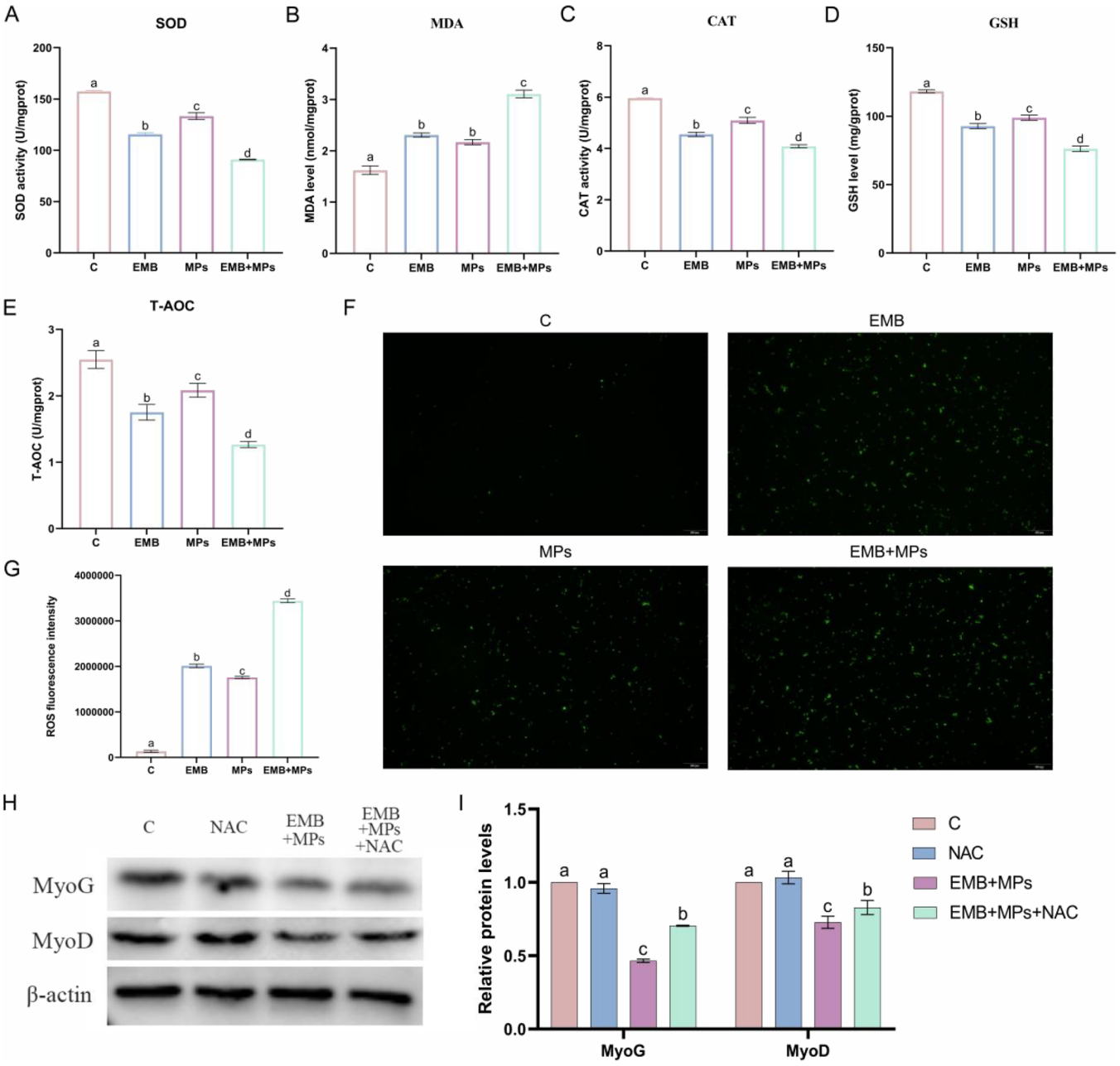

Figure 3. Exposure to EMB and/or MPs exacerbated oxidative stress and induces excessive production of ROS. (A–E) The effects of exposure to EMB and/or MPs on the activities of SOD, CAT, and T-AOC as well as the levels of GSH and MDA. (F, G) ROS fluorescence staining and quantitative analysis results of CCM cells. (H, I) Protein levels and quantitative analysis results of indicators related to myogenic differentiation after NAC treatment.

of exposure to EMB and MPs on protein synthesis and degradation showed that UPS and ALS were significantly activated after exposure to EMB and/or MPs. IF staining (Figure 5A–F) and protein levels (Figure 5G–J) detection results were shown in the figures, and MAFbx, MURF1, LC3B, and BNIP3 levels were markedly elevated ($P < 0.05$). However, the protein synthesis pathway showed the opposite trend with significantly reduced protein levels of IGF-1, p-AKT, and p-p70S6K (Figure 5K–N). These results indicated that exposure to EMB and/or MPs resulted in skeletal muscle atrophy and decreased muscle quality by inhibiting the IGF-1/AKT protein synthesis pathway and promoting the degradation pathways of UPS and ALS proteins.

4. DISCUSSION

EMB is a widely used insecticide, but long-term widespread use of drugs can also lead to excessive residues in food, causing environmental pollution and endangering the health of fish. Previous studies have found that insecticides and environmental pollutants may coexist geographically, such as EMB and MPs, both of which can cause damage to multiple tissues and organs.[44] Based on this, this study investigated the influence of EMB and/or MPs exposure on common carp skeletal muscle and the potential mechanisms involved. In this research, exposure to EMB and/or MPs caused skeletal muscle atrophy in common carp and induced oxidative stress, mitochondrial dysfunction, and protein synthesis and degradation imbalance.

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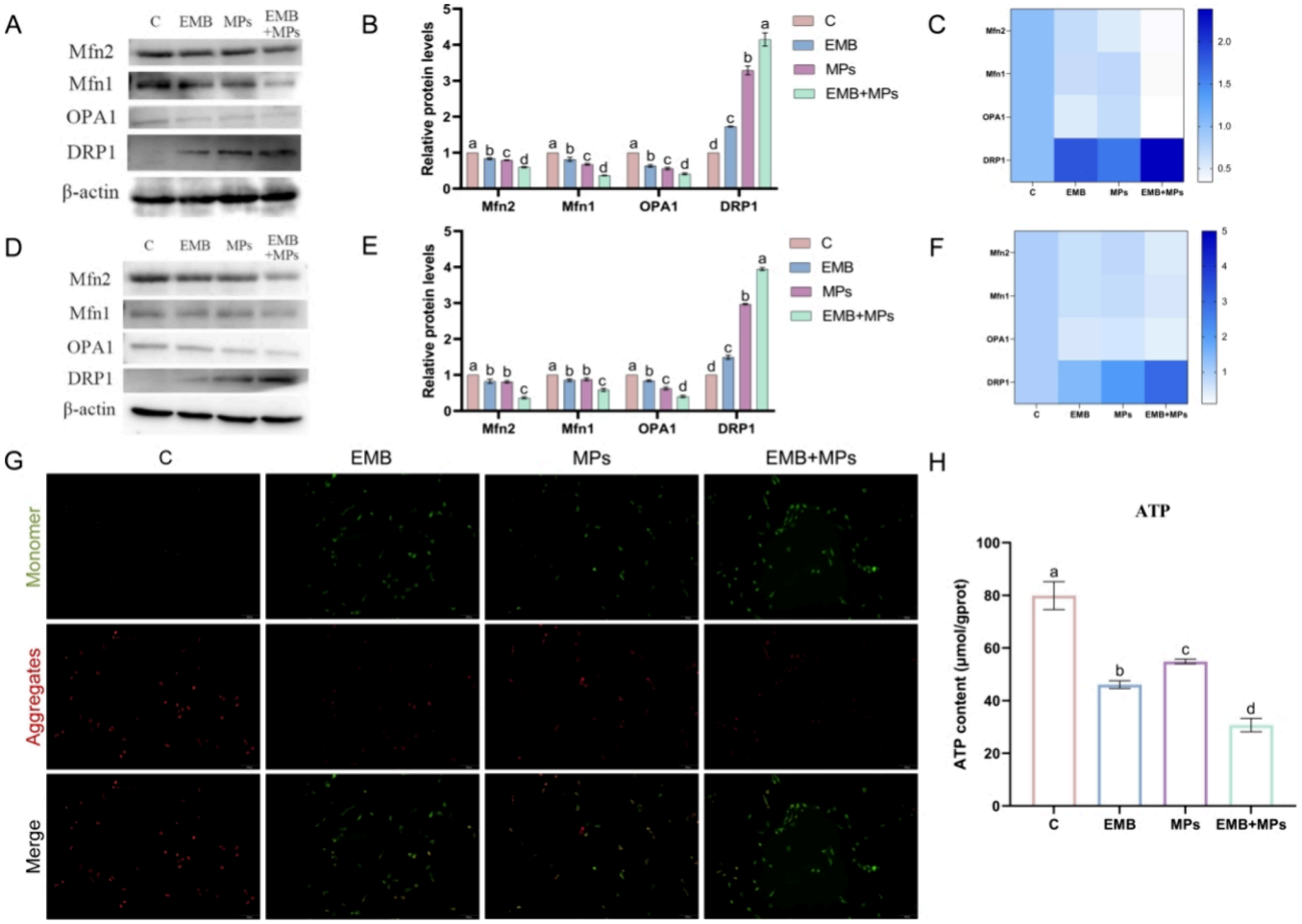

Figure 4. Exposure to EMB and/or MPs induced mitochondrial dysfunction. (A–F) Protein levels and quantitative analysis results of mitochondrial fusion and division related indicators in skeletal muscle and CCM cells as well as mRNA expression. (G) Measurement results of mitochondrial membrane potential in CCM cells. (H) The ATP content detection results of skeletal muscle.

Moreover, the coexposure of EMB and MPs exacerbated the atrophy of common carp skeletal muscles, while the addition of NAC could alleviate the skeletal muscle atrophy induced by their coexposure.

Oxidative damage is closely related to the cytotoxic processes of various environmental pollutants. For example, pyrethroid insecticides such as deltamethrin, cypermethrin, and lambda cyhalothrin triggers oxidative stress in fish tissues, specifically the gills, liver, and muscle, causing tissue damage and further inducing DNA damage and cell apoptosis.[45] Acrylamide can result in a lessening of enzymatic activity of SOD and GST as well as an escalation in ROS levels, inducing oxidative stress and developmental toxicity in zebrafish, resulting in cardiovascular system defects, severe cardiac malformations, and functional impairments.[46] Exposure to EMB induces mitochondrial dysfunction by triggering oxidative stress, promoting autophagy and ferroptosis in kidney cells, ultimately leading to kidney damage in carp.[47] Moreover, MPs can also increase the level of MDA, decrease the activity of antioxidant enzymes such as CAT, SOD, and GSH-Px, and cause apoptosis and necrotic apoptosis in mouse skeletal muscle cells.[48] Our research similarly confirmed that exposure to EMB and/or MPs increased ROS accumulation, increased the MDA level, and decreased SOD, CAT, T-AOC activity, and GSH level. Meanwhile, oxidative stress can also lead to dysfunction of organelles in cells, especially mitochondria. For example, cadmium-induced oxidative stress further causes mitochondrial damage and a decrease in mitochondrial membrane potential in BEAS-2B cells.[49] Exposure to ultrafine black carbon in the environment induces oxidative stress in SH-SY5Y cells, giving rise to mitochondrial dysfunction and autophagy.[50] Mitochondria are the core organelles of skeletal muscle metabolism, and exposure to arsenic trioxide (ATO) causes significant mitochondrial damage and dysfunction, including mitochondrial vacuolization and mitochondrial fusion/fission imbalance, leading to duck skeletal muscle injury.[51] Diquat causes skeletal muscle atrophy in piglets by disrupting mitochondrial morphology and promoting protein degradation in muscle tissue.[52] In this research, exposure to EMB and/or MPs resulted in mitochondrial dysfunction, manifested as decreased mRNA expression and protein levels of mitochondrial fusion proteins (Mfn1, Mfn2, and OPA1), increased mRNA expression and protein levels of mitochondrial fission proteins (DRP1), decreased mitochondrial membrane potential, and decreased ATP content. The research findings imply that exposure to EMB and/or MPs generated oxidative stress, leading to mitochondrial dysfunction, and their coexposure exacerbated the occurrence of oxidative stress and mitochondrial dysfunction. In addition, the addition of NAC to CCM cells confirmed that EMB and MPs participate in the process of skeletal muscle atrophy by regulating the ROS.

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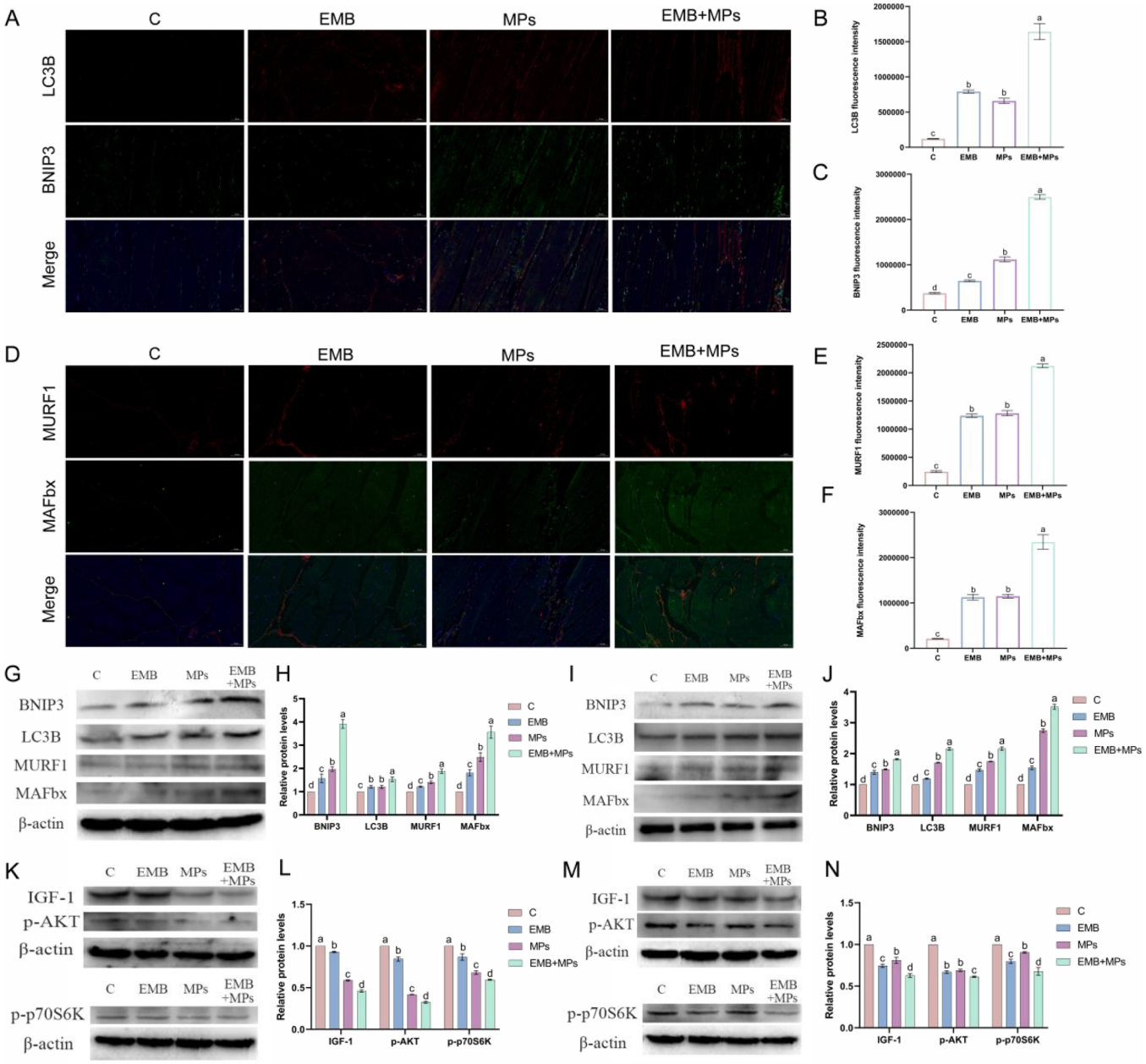

Figure 5. Exposure to EMB and/or MPs disrupted the balance between protein synthesis and degradation. (A−C) LC3B and BNIP3 IF staining (20×) and quantitative analysis results of skeletal muscle tissue, bar = 50 μm. (D−F) MAFbx and MURF1 IF staining (20×) and quantitative analysis results of skeletal muscle tissue, bar = 50 μm. (G−J) Relative protein levels of protein degradation related indicators in skeletal muscle tissue and CCM cells. (K−N) Relative protein levels of protein synthesis related indicators in skeletal muscle tissue and CCM cells.

The equilibrium of protein production and degradation is vital for maintaining skeletal muscle health, and muscle atrophy occurs when the protein synthesis rate decreases, relative to the protein degradation rate. Insulin and insulin-like growth factor-1 (IGF-1) are crucial positive regulatory factors in protein synthesis, which induce phosphorylation of ribosomal protein S6 (RPS6) through Akt phosphorylation, a critical event in protein translation.[53] For example, Zhimu and Huangbai herb pair can reverse cancer cachexia muscle atrophy by activating IGF-1/Akt signaling.[54] IGF-1/Akt is also crucial for maintaining the quality of chicken breast meat.[55] P70 ribosomal protein S6 kinase (p70S6K) is a serine/threonine protein kinase whose phosphorylation initiates protein translation processes, the sharp decrease in p70S6K caused by Acetyltanshinone IIA (ATA) treatment leads to a reduction in the synthesis of several cell cycle related proteins in drug-resistant lung cancer cells.[56] UPS and ALS are two major protein degradation systems, both involved in maintaining protein homeostasis and continuously adjusting the proteome to maintain cellular function. Inhibition of UPS and ALS can inhibit muscle protein degradation and alleviate muscle atrophy in mice.[57] In this experiment, exposure to EMB or MPs induced significant activation of skeletal muscle UPS and ALS. The protein levels of MAFbx, MURF1, LC3B, and BNIP3 were markedly increased, while the protein synthesis pathway showed the opposite trend. The protein levels of IGF-1, p-AKT, and p-p70S6K were significantly decreased. The outcomes reveal that exposure to EMB and/or MPs promoted

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UPS and ALS protein degradation pathways and inhibited the IGF-1/AKT protein synthesis pathway, leading to skeletal muscle atrophy and ultimately resulting in decreased muscle quality.

In summary, this experiment demonstrates a new potential mechanism of skeletal muscle atrophy in common carp induced by exposure to EMB and/or MPs. Exposure to EMB or MPs led to excessive ROS production, resulting in mitochondrial dysfunction and an imbalance in skeletal muscle protein synthesis degradation, ultimately leading to skeletal muscle atrophy in common carp. The above changes caused by the coexposure of the two were significantly stronger than those caused by a single exposure. In addition, the NAC treatment can effectively alleviate skeletal muscle atrophy. This study is the first to discover the potential toxic mechanism of EMB-induced skeletal muscle atrophy in carp, assisting in the enhanced comprehension of the connection between EMB and MPs exposure and health issues affecting animals and humans and also has certain guiding significance for actual water resources and the environment protection.

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Notes

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