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Selenium deficiency inhibits micRNA-146a to promote ROS-induced inflammation via regulation of the MAPK pathway in the head kidney of carp

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ABSTRACT

Selenium (Se) is a necessity in multiple species of fish. Se plays an important role in immunoregulation, inflammation, and antioxidant systems in fish and other animals. The head kidney is the major immune organ in adult carp, and it produces white blood cells and destroys old red blood cells. The present study aimed to explore the effects and regulatory molecular mechanisms of Se on ROS and micRNA-146a as part of the inflammatory response in fancy carp. Adult fancy carp were fed different concentrations of Se in their diets. The Se content of the head kidney changed in a pattern consistent with the dietary content of Se. Se deficiency induced a significant increase in ROS, restrained the activities of GPx, SOD and CAT and increased MDA content. qPCR analysis showed a reduction in micRNA-146a with Se deficiency. The Se content, miRNA-146a expression and ROS levels were correlated. H_2O_2 cell stimulation assays found that ROS could activate the MAPK pathway, and ELISA results showed p38, JNK and ERK phosphorylation significantly increased with H_2O_2 stimulation. TNF-α, IL-1β, and IL-6 were appreciably increased. At same time, miRNA-146a, which should have increased to regulate the inflammatory response, was reduced with Se deficiency. Therefore, with Se deficiency, the head kidney was inflamed. All these results indicated that Se deficiency inhibits micRNA-146a to promote ROS-induced inflammation via regulating the MAPK pathway in the head kidney of carp. The present study revealed that supplementing the diet of carp with selenium is beneficial for growth and disease prevention.

1. Introduction

Selenium (Se) is an important micronutrient in both aquatic animals and others [1,2]. Se is important and effects immune organs, nervous tissue, the reproductive system, muscle tissue and a variety of other physiological activities [3–5]. Se participates in immune defense against endogenous or exogenous stimuli, which promotes the healthy growth of animals [6]. It has been verified that Se plays an important role in immunoregulation and inflammation, and these physiological responses are closely associated with dietary Se intake [7]. The necessity and requirements of Se have been estimated in multiple species of fish [8]. The previous study have shown that Se regulates inflammatory through the HSP60-TLR2-MAPKs signaling pathway in carp [9]. Furthermore, Se is an essential nutrient in antioxidant systems in fish and other animals. GPx was the earliest known bioactive form of Se. Other antioxidase activities were affected by Se [10]. These antioxidase enzymes help the animal remove free radicals, reducing unnecessary damage caused by the free radicals [11].

Reactive oxide species (ROS) are the major free radical in the body. Although ROS play an important role in the process of immune defense, excessive production of ROS may induce adverse reactions in the body, such as inflammation and apoptosis [12,13]. The deficiency of Se can lead to an increase in the production of ROS in various tissues. It was reported in our previous study that Se deficiency induces and exacerbates inflammatory injury in carp [9]. ROS can act as an inducer to activate inflammatory signaling pathways [14]. Whether ROS play a role in Se-induced inflammation is unclear, especially in fish.

MicroRNA (micRNA) is a class of noncoding, small molecule, single-stranded RNA involved in immune defense, apoptosis, free radical injury and other biological processes [15]. micRNA-146a is a typical multifunctional micRNA molecule, which has become a key transcription regulator for inflammatory related diseases in inflammation in response to both endogenous and exogenous stimuli [16,17]. micRNA-146a has been proven to manipulate TLRs and the downstream inflammatory signaling pathways in order to modulate the inflammatory process in otitis media, chronic periodontitis and other diseases

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[18–20]. There is evidence that micRNA-146a increases oxidative stress in fish [21]. It was also found the micRNA-146a is downregulated in VE-deficient tilapia [22]. It may be concluded that micRNA-146a is associated with oxidative stress in fish.

It has been reported that multiple micRNAs are regulated by Se, including micRNAs such as micRNA-374, micRNA-16, micRNA-199a, micRNA-155 and micRNA-30e [23]. Se has shown an effect on inflammation by regulating the expression of micRNA-146a and the MAPK pathway [23]. However, there is no report about the interconnections of Se deficiency induced inflammation, ROS and micRNA-146a in fish. The present study was carried out to explore the effects and regulatory molecular mechanisms of Se on ROS and micRNA-146a in the inflammatory response of fancy carp.

2. Materials and methods

2.1. Animals and treatment groups

Sixty adult fancy carp (body weight 2 ± 0.03 kg, length 50 ± 0.17 cm) were purchased from a Aquarium market. The carp were randomly distributed in six laboratory tanks (2.0 × 2.0 × 0.8 m) at a photoperiod of 12-h light/12-h dark, water temperature at 23 ± 0.5 °C, dissolved oxygen level above 8 mgL^{-1}. The carp diet was only made with corn oil and starch (contain 0.013 mg of Se/kg), obtaining from Jilin, a Se-deficiency region in China. Organic Se (selenomethionine) was supplemented at 0, 0.7, and 1.5 mg Se/kg-diet. The diet Se ultimate concentrations were measured as 0.015, 0.80, and 1.49 mg Se/kg-diet at last. One-third of tank water was changed daily. All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee of Hubei University. The craps were divided into three groups (20 fish in two tank) as follows: a) Se Deficiency (SeD): the fish were fed 0.015 mg Se/kg-diet. b) Se Normal (SeN): the craps were fed 0.80 mg Se/kg-diet. c) Dietary Se Supplements (SeS): the craps were fed 1.49 mg Se/kg-diet. After 140 days of feeding, the craps were anaesthetized with tricaine methane sulphonate buered with 0.4 g/L $NaHCO_3$. The head kidney was harvested for the follow studies.

2.2. Tissue Se concentration assay

The Se concentration of head kidney tissues was on anatomic fluorescence spectrophotometer. In acidic environment, the fluorescence substance is generated by Se and 2,3-2 amino naphthalene (DAN) with cyclohexane extracte. The head kidney tissues were converted to selenous acid by concentrated nitric acid and perchloric acid for digestion, evaporated completely at 160 °C. Concentrated hydrochloric acid was added, which reduced the hexavalent selenium to quadrivalent Se. the excitation wavelength and the fluorescence wavelength was used to determine the Se concentration.

2.3. ROS detection

Head kidney tissues were homogenized on ice and then made into a single cell suspension. 2′,7′- Dichloro fluorescein yellow diacetate was added to the suspension, which was then incubated at 37 °C for 1 h, centrifuged for 10 min, collected as single cells, and washed in a PBS solution. ROS levels were measured with a kit in accordance with the manufacturer's instructions (Nanjing Jiancheng BIO, Inc, China). An excitation wavelength of 500 nm and fluorescence wavelength of 525 nm were used to determine the quantity of ROS.

2.4. Antioxidant enzyme activity analysis

Head kidney tissues were homogenized on ice. The cells were lysed with an ultrasonic cytometer. The supernatant was removed after centrifugation at 3000 r/min and tested. The GPx activity was measured with oxidized glutathione to show. GPx was catalytically reduced to GSH and H_2O_2, GSH was oxidized into oxidized glutathione, and the mixture was measured with a spectrophotometer at 340 nm at 37 °C. The photometric value was measured at the wavelength. All operations followed the manufacturer's instructions. The SOD activity was measured with the inhibition and form of formazan, and the mixture was measured with a spectrophotometer at 505 nm at 37 °C. The CAT activity was measured as the consumption rate of H_2O_2, and the mixture was measured with a spectrophotometer at 230 nm at 37 °C. The MDA content was measured with thiobarbituric acid incubated at 95 °C. When the pink reaction was observed, the mixture was measured with a spectrophotometer at 532 nm. The unit of MDA content was nmol/mg protein.

2.5. Cell isolation and culture

Macrophage cells from the head kidney were prepared. The head kidney cells were aseptically harvested, minced into paste, and then made into a single cell suspension. Physiological osmotic pressure Percoll was diluted by 0.7% NaCl until reaching 34% or 51%. The following were added in order to a centrifuge tube: 51% Percoll, single cell suspension, and 34% Percoll. The macrophages were found between two Percoll of solution. The cells were collected and resuspended in RPMI-1640 containing 10% FCS, incubated for 4 h at 37 °C, and cleared of fibroblasts. The medium was changed to serum-free medium after 24 h. Macrophages were incubated with selenomethionine (0, 1, and 10 μM) as the Se Deficiency (SeD), Se Normal (SeN), and Selenium Supplement (SG) treatments, respectively, for 24 h at 37 °C in 5% CO_2 in six-well plates.

2.6. ELISA assays of inflammatory factors and pathway

The head kidney tissues were homogenized with PBS on ice and centrifuged at 2000 g for 30 min at 4 °C, and then the supernatants were collected. The macrophage of head kidney culture supernatants were collected. All the supernatants were assayed for TNF-α, IL-1β, IL-6, levels using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (BioLegend, Inc, Camino Santa Fe, Suite E, San Diego, CA, USA). All the supernatants were also assayed MAPK pathway proteins, p38, ERK and JNK were analyzed by the corresponding ELISA kits (BioLegend, Inc., Camino Santa Fe, Suite E, San Diego, CA, USA) according to manufacturer's instructions. The experiments were performed thrice.

2.7. Quantitative real-time polymerase chain reaction (qPCR)

The head kidney were homogenized. The macrophage of head kidney with different treatment were collected. The total RNA was isolated from the tissues and cells using TRIzol according to the manufacturer's instructions (Invitrogen, China). The RNA concentration and purity were determined spectrophotometrically at 260/280 nm and then reverse transcribed into cDNA. The primers used to amplify specific genes are designed by software Primer 5.0. The primers for micRNA-146a was Anti-sense-GCATGCGGTTAACTAAT and Sense-GTGCAGGGTCCGAGGT, adding Stem-loop CTCAACTTCGTGGAGTCG GCAATTCAGTTGAGACCCCTAT. The primers were used for the detection of p38, ERK, JNK, TNF-α, IL-1β, IL-6, and β-actin in Table 1. A 25-μl reaction was performed, 16 μl of 2 × SYBR Green I PCR Master Mix (TaKaRa, China), 2 μl of diluted cDNA, 1 μl of each primer (10 μM), 1.6 μl of 50 × ROX reference Dye II, and 3.4 μl of PCR-grade water on an ABI PRISM 7500 Detection System (Applied Biosystems, USA). The results were expressed as $2^{-\Delta\Delta Ct}$. β-actin was used as control.

2.8. Statistical analyses

The SPSS statistical software for Windows (version 13; SPSS Inc.,

Table 1
Primers used for quantitative real-time PCR.

Name	Primer sequence	Product size (bp)
p38	Sense: 5′- GTTGATGTTCAGGCTGTG-3′ Anti-sense:5′- GACGGTAGACATTCTGGTAG-3′	209
ERK	Sense: 5′- AAAATCAAAGAAGCCTACTCCC-3′ Anti-sense: 5′- TTCCCAACTCCTGCGACA-3′	214
JNK	Sense: 5′- CATTCGTTGTTGCCTTGC-3′ Anti-sense:5′- TGTAGTGGGCGTCCTGTG-3′	225
TNF-α	Sense: 5′- ATAACCAGATCGTGATCCCACA -3′ Anti-sense: 5′- TTCGCCCTTCCGACCTCA -3′	195
IL-1β	Sense: 5′- GCCAGACCTGTAGCCCTAG -3′ Anti-sense: 5′- TGTCCGTGCTGATGAACC -3′	229
IL-6	Sense: 5′- ACAGTTTGTGGAGGAGTT -3′ Anti-sense:5′- GGAGTAGGGTTGATTGAG -3′	209
β-actin	Sense: 5′- CCATCGTCCACCGCAAAT -3′ Anti-sense:5′- CGCCTCCTTCATCGTTCC-3′	207

Chicago, IL, USA) was used in present study for the statistical analysis. The data of cytokine assays, protein levels and mRNA levels, which were expressed as the means ± SD, were assessed using the Tukey-Kramer method for multiple comparisons. The significance was determined with one-way analysis of variance at a significance level of $p < 0.05$.

3. Results

3.1. Se concentrations in the head kidney

Se concentration was affected by dietary Se content (Fig. 1A). Compared to the normal group (SeN), the Se concentration was significantly reduced in the Se deficiency diet group (SeD). As Se concentrations increased in the diet, the Se concentration increased remarkably. Compared to the normal group (SeN), the Se concentrations of head kidney were increased markedly in the SeS group.

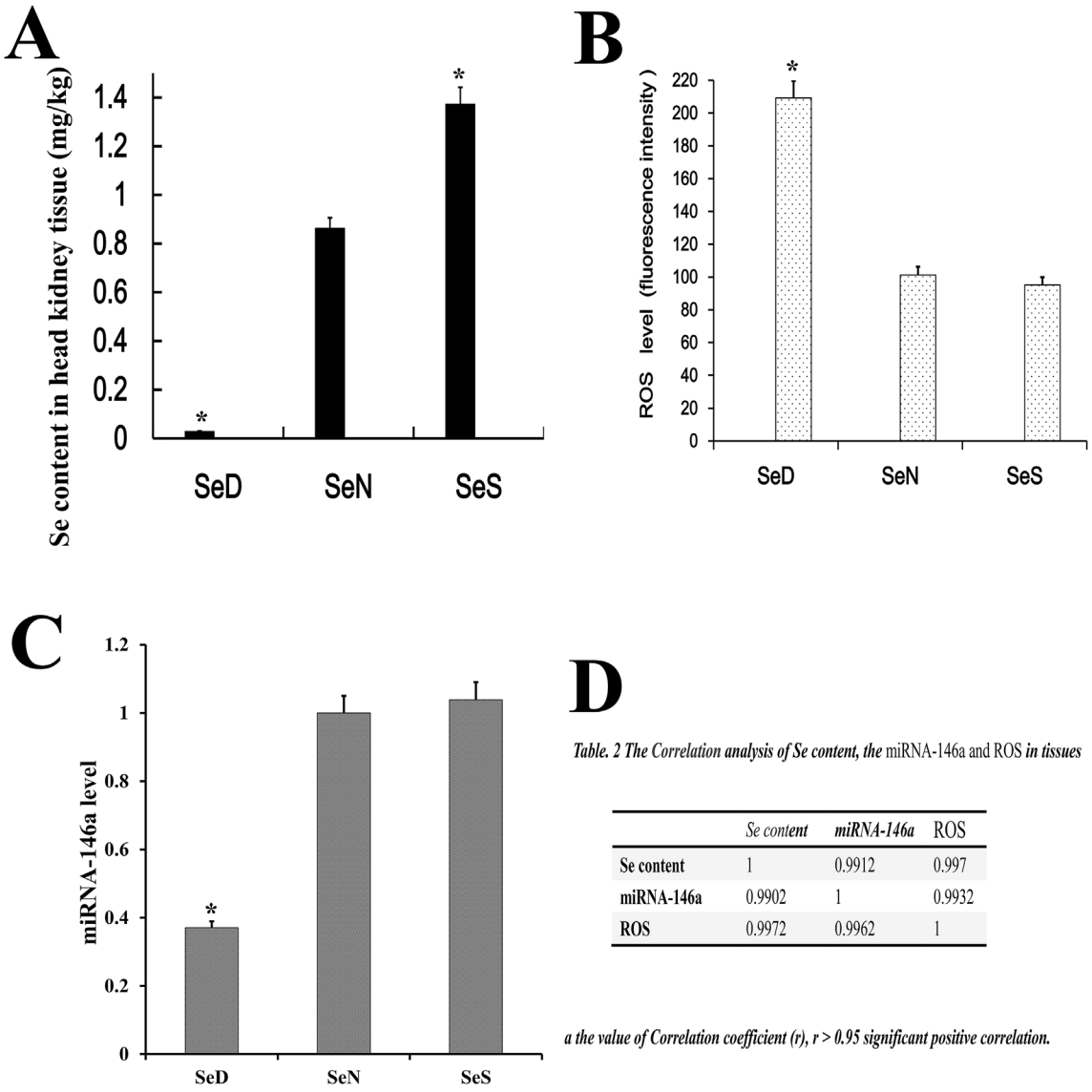

Fig. 1. Se concentrations, ROS level and micRNA-146a correlation analysis. (A) Se concentrations in head kidney with different diets. (B) The ROS level in head kidney tissues. (C) The micRNA-146a level in head kidney tissues. (D) The correlation analysis of Se content, the miRNA-146a and ROS in tissues. SeD, the fish fed a dietary Se deficiency at 0.015 mg Se/kg-diet. SeN, the fish fed normal dietary Se at 0.80 mg Se/kg-diet. SeS, the fish fed dietary Se supplements at 1.49 mg Se/kg-diet. Data are presented as the means ± SD. (n = 10 per group). *$p < 0.01$ indicates a significant difference from the SeN fish.

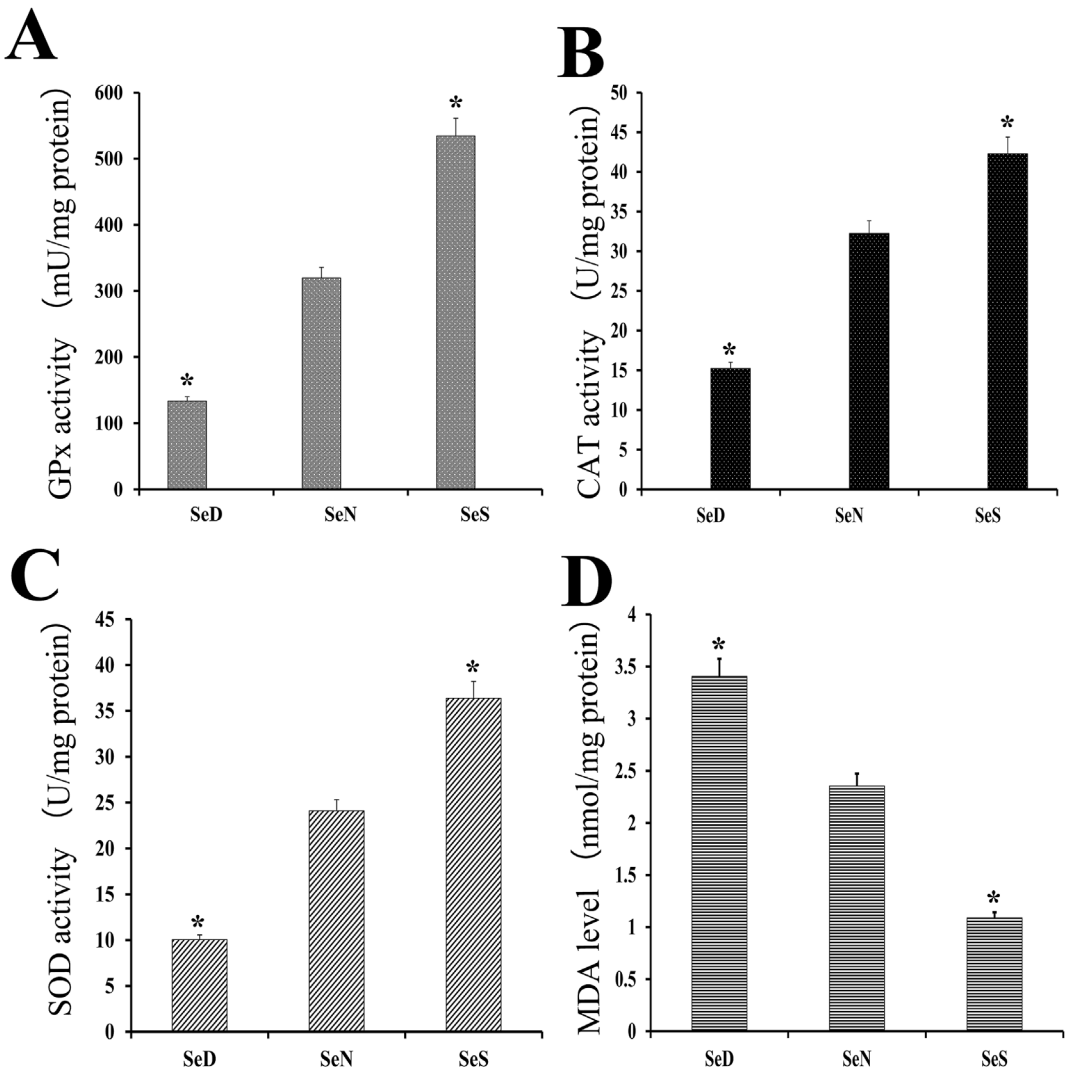

Fig. 2. The autioxidant enzyme activities analysis in head kidney tissue. (A) The GPx activities in head kidney tissues. (B) The CAT activities in head kidney tissues. (C) The SOD activities in head kidney tissues. (D) The MDA level in head kidney tissues. SeD, the fish fed a dietary Se deficiency at 0.015 mg Se/kg-diet. SeN, the fish fed normal dietary Se at 0.80 mg Se/kg-diet. SeS, the fish fed dietary Se supplements at 1.49 mg Se/kg-diet. Data are presented as the means ± SD. (n = 10 per group). *$p < 0.01$ indicates a significant difference from the SeN fish.

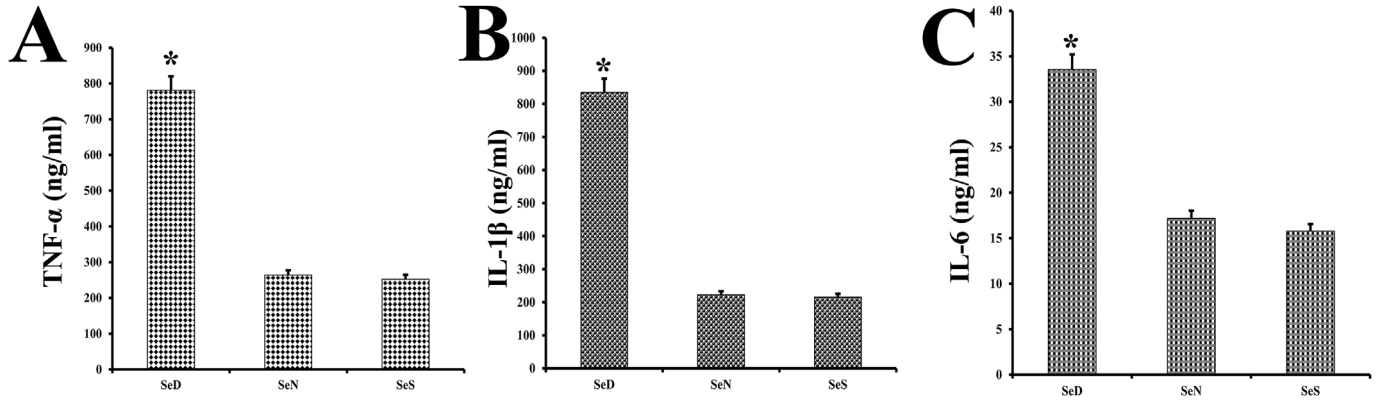

Fig. 3. The productions of inflammatory factors in head kidney tissue. (A) The TNF-α production levels in head kidney tissues. (B) The IL-1β production levels in head kidney tissues. (C) The IL-6 production levels in head kidney tissues. SeD, the fish fed a dietary Se deficiency at 0.015 mg Se/kg-diet. SeN, the fish fed normal dietary Se at 0.80 mg Se/kg-diet. SeS, the fish fed dietary Se supplements at 1.49 mg Se/kg-diet. Data are presented as the means ± SD. (n = 10 per group). *p < 0.01 indicates a significant difference from the SeN fish.

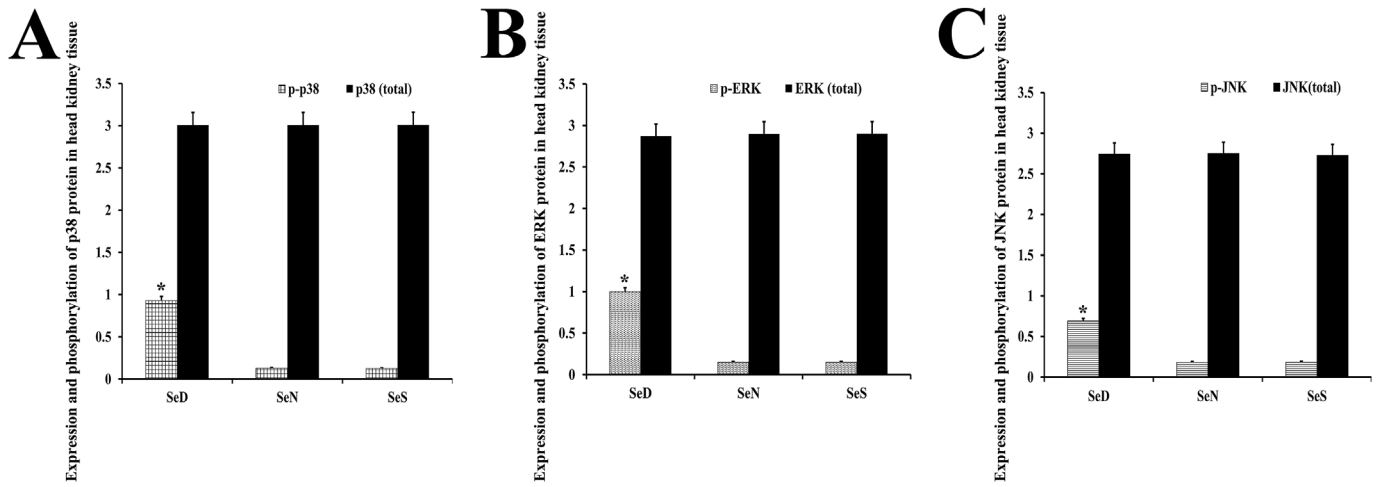

Fig. 4. The MAPKs pathway activation in head kidney tissues. (A) The p38 protein and phosphorylation levels in tissues. (B) The ERK protein and phosphorylation levels in tissues. (C) The JNK protein and phosphorylation levels in tissues. SeD, the fish fed a dietary Se deficiency at 0.015 mg Se/kg-diet. SeN, the fish fed normal dietary Se at 0.80 mg Se/kg-diet. SeS, the fish fed dietary Se supplements at 1.49 mg Se/kg-diet. Data are presented as the means ± SD. (n = 10 per group). *p < 0.01 indicates a significant difference from the SeN fish.

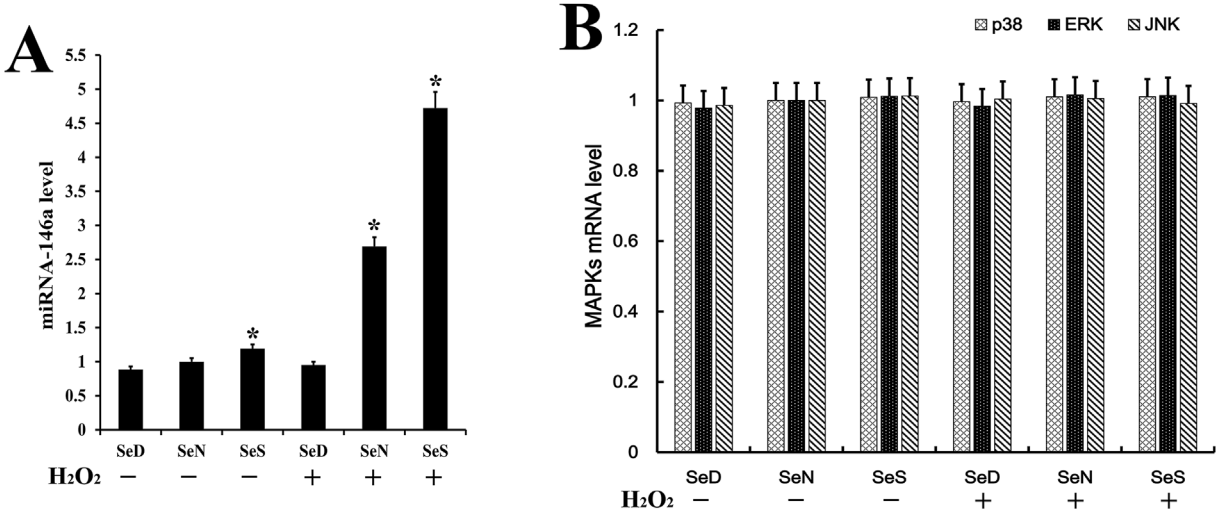

Fig. 5. Influence of oxygen radical on micRNA-146a and MAPKs mRNA level in macrophage. (A) The mRNA levels of micRNA-146a with H_2O_2 stimulating in macrophage. (B) The mRNA levels of MAPKs with H_2O_2 stimulating in macrophage. β-actin was used as a control. SeD, incubated with selenomethionine 0 μM. SeN, incubated with selenomethionine 1.0 μM. SeS, incubated with selenomethionine 10 μM. Data are presented as the means ± SD. (n = 10 per group). *p < 0.01 indicates a significant difference from the SeS cells.

3.2. The ROS level in head kidney

To analyze the effect of Se on tissue free radical production, ROS were measured. The results are shown in Fig. 1B. Under normal circumstances, there was some generation of ROS. When the Se concentration was significantly reduced, ROS growth was rapid. Compared to the normal group (SeN), ROS significantly increased in the SeD group and decreased slightly in the SeS group.

3.3. Effects of dietary Se on micRNA-146a expression and correlation analysis

micRNA-146a was affected by Se concentration in the tissues. Expression of micRNA-146a significantly decreased in the SeD group, and a slight increase was found in the SeS group (Fig. 1C). The changes were similar to the effects of ROS, but with the opposite trend. Correlation analysis of Se content, miRNA-146a expression and ROS levels was performed in the tissues. The results displayed a clear correlation (Fig. 1D).

3.4. The antioxidant enzyme activity analysis

The scavenging of free radicals was dependent on antioxidase. Antioxidant enzyme activities were regulated and controlled by Se. In present study, four antioxidases were analyzed. The results are shown in Fig. 2. The GPx was a Se enzyme. With changing Se concentration, GPx activity shown consistent changes (Fig. 2A). GPx activity was markedly decreased in the SeD group and significantly increased in the SeS group. The other two enzymes, CAT and SOD, were also detected. These enzymes also showed similar trends to GPx. Compared to the normal group, the CAT and SOD activities were significantly decreased in the SeD group but significantly increased in the SeS group (Fig. 2B and C). To further confirm the corresponding effects, MDA was measured. The results showed that MDA was markedly increased in the SeD group. With the increase in the Se concentration, MDA showed a decreasing trend (Fig. 2D).

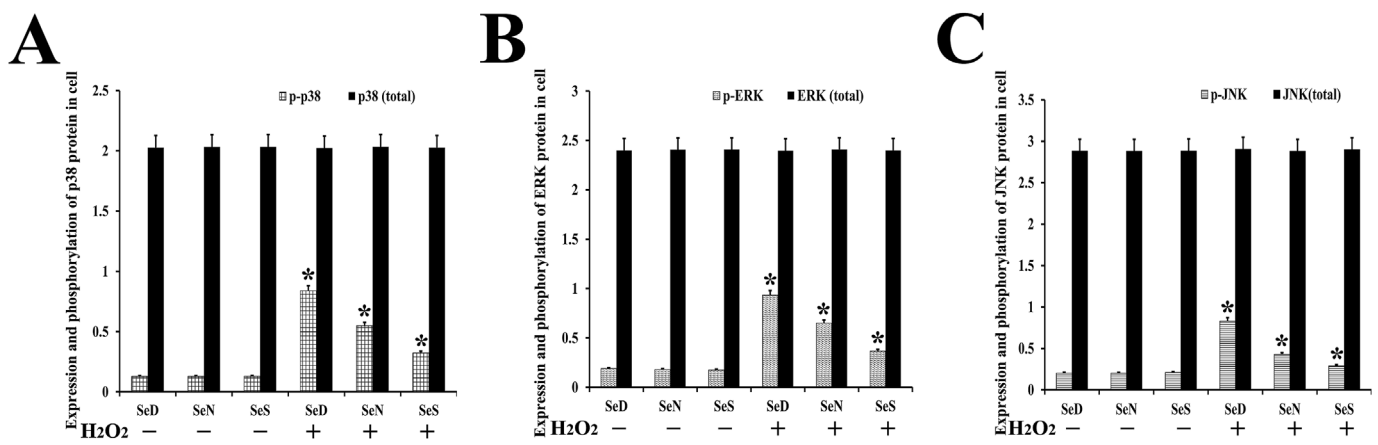

Fig. 6. The MAPKs pathway activation in macrophage. (A) The p38 protein and phosphorylation levels in macrophage with or without H_2O_2 stimulating. (B) The ERK protein and phosphorylation levels in macrophage with or without H_2O_2 stimulating. (C) The JNK protein and phosphorylation levels macrophage with or without H_2O_2 stimulating. SeD, incubated with selenomethionine 0 μM. SeN, incubated with selenomethionine 1.0 μM. SeS, incubated with selenomethionine 10 μM. Data are presented as the means ± SD. (n = 10 per group). *p < 0.01 indicates a significant difference from the SeS cells.

3.5. Effects of dietary Se on inflammatory factor production in head kidney tissues

Head kidney tissues were homogenized, and the supernatants were collected. The pro-inflammatory factors, TNF-α, IL-1β and IL-6, were detected. The results are shown in Fig. 3A–C. TNF-α, IL-1β, and IL-6 were appreciably increased in the SeD group. There was no significant increase in both the SeS and SeN groups.

3.6. Effects of dietary Se on the MAPK pathway in head kidney tissues

The MAPK pathway plays an important role in the regulation of inflammatory factors. The biological regulation of these proteins is accomplished by protein phosphorylation. The expression and phosphorylation of p38, JNK and ERK were assessed in head kidney tissues and no abnormal changes were observed, but the phosphorylation of p38, JNK and ERK varied significantly. The phosphorylation was noticeably increased in the SeD group. No significant difference was found between the carp in the SeS and SeN groups (Fig. 4D).

3.7. Influence of oxygen radicals on micRNA-146a and MAPK mRNA levels in the macrophages of the head kidney

To further corroborate the findings of the effects of ROS, macrophages of the head kidney were separated in culture and then a H_2O_2 stimulus was applied. micRNA-146a and MAPKs mRNA levels were assessed using qPCR analysis (Fig. 5A). The results showed that micRNA-146a significantly increased with H_2O_2 stimulus. The increase was very weak in the SeD treatment. The increase was very strong in the SeS and SeN groups, especially in the SeS group. The expression of p38, JNK and ERK mRNA levels was not different under the different treatment conditions. No difference was found in the Se feeding groups at different concentrations (Fig. 5B).

3.8. Effects of oxygen radicals on the expression and phosphorylation of MAPKs in the macrophages of the head kidney

ROS can activate the MAPK signaling pathway to induce inflammation. No difference was seen at the genetic level, so the expression and phosphorylation were detected. The results were similar to those of the tissue test. The H_2O_2 stimulus induced a significant increase in p38, JNK and ERK phosphorylation (Fig. 6). With H_2O_2 stimulus, p38, JNK and ERK phosphorylation were significantly increased, when compared with the SeN treatment. However, the phosphorylation increased was significantly reduced in the SeS group (Fig. 6). These results further supported the idea that ROS played a regulatory role in the process of Se deficiency induced inflammation.

3.9. Effects of oxygen radicals on the inflammatory factors in the macrophages of the head kidney

To confirm that the regulation of ROS induces inflammatory responses, the pro-inflammatory factors were analyzed in the macrophages of the head kidney. The TNF-α, IL-1β and IL-6 mRNA levels were assessed using qPCR analysis (Fig. 7A–C). The results showed the TNF-α, IL-1β and IL-6 mRNA was significant increased with the H_2O_2 stimulus. The increase was very strong in the SeD, SeS and SeN groups, especially in the SeD group. With the increase in the Se concentration, the increasing trend was gradually weakened, but still remained at a high level. The TNF-α, IL-1β and IL-6 proteins were also analyzed by ELISA (Fig. 7D–F). The results were consistent with the results of genetic testing. With the H_2O_2 stimulus, TNF-α, IL-1β and IL-6 production was significantly increased when compared with the SeN group, but the increase was significantly reduced in the SeS group (Fig. 7D–F).

4. Discussion

Some cyclostomes and bony fish still retain the residual anterior kidney, which is known as the head kidney, in the adult. The head kidney is at the front of each kidney in carp. The surface of the head kidney is covered with a thin layer of fibroid connective tissue capsule. The head kidney is the major immune organ in carp [24], producing white blood cells and destroying old red blood cells. It has been verified that Se plays an important role in immunoregulation and inflammation and is closely associated with dietary Se intake [7]. Se deficiency can cause immune deficiency, inhibit growth [25], and induce oxidative stress in fish [26,27]. In present study, the head kidney was the same as the other tissues, the Se concentration was affected by dietary Se content. As the dietary Se concentrations increased, the Se concentration was remarkably increased in the head kidney. Se deficiency will induce inflammation and reduce antioxidant capacity [10,11]. The effective scavenging of ROS can be used as a standard way to evaluate antioxidant capacity [28]. In the present study, there was some generation of ROS under normal circumstances. When the Se concentration was significantly reduced, ROS induction was rapidly increased. This observation was the same pattern as in a previous study in which Se deficiency induced an ROS excessive agglomeration [29].

Although ROS play an important role in the process of immune defense, excessive production of ROS may induce adverse reactions in the body, such as inflammation and apoptosis [12,13]. The scavenging

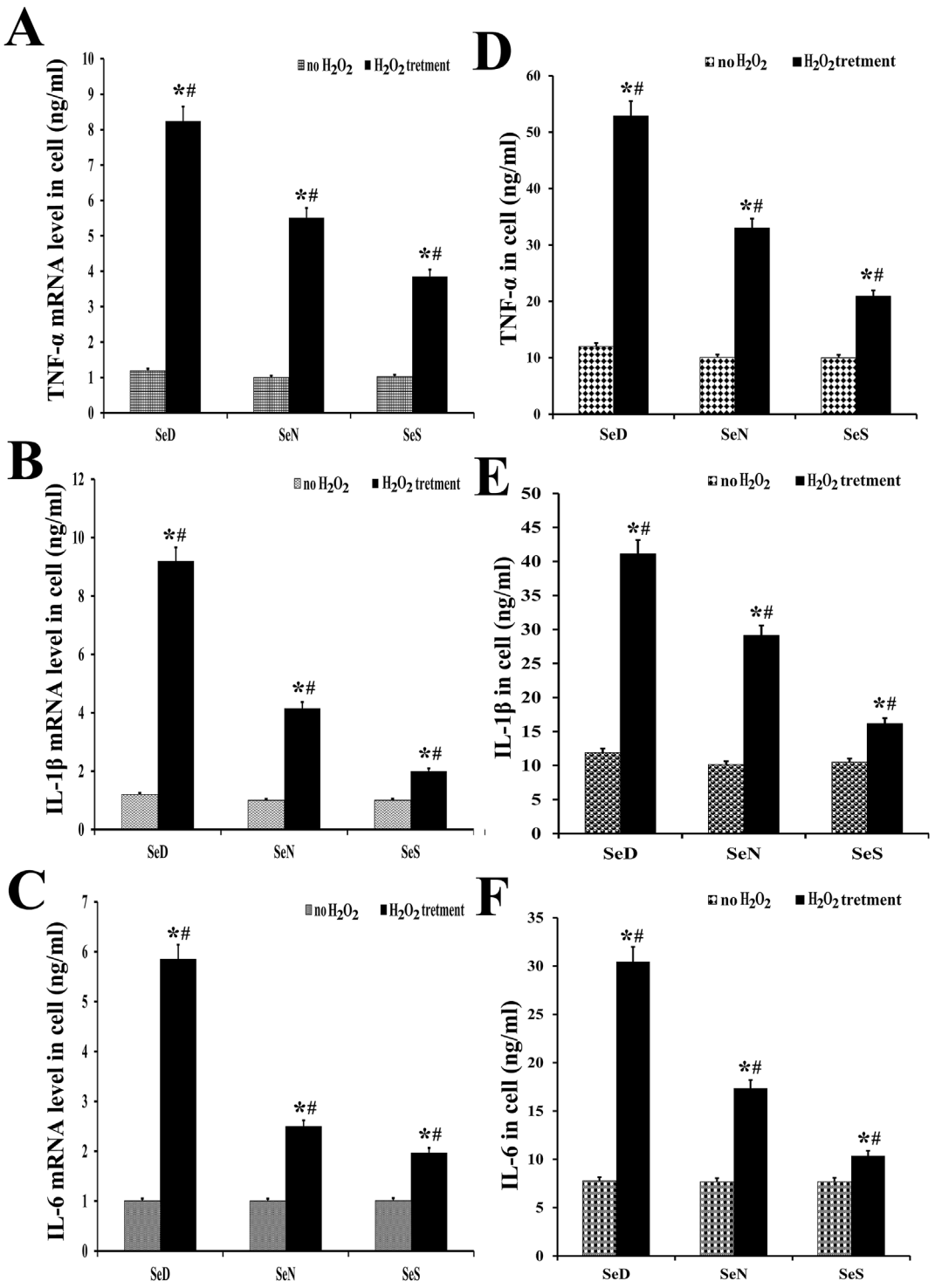

Fig. 7. The expressions of inflammatory factor in macrophage. (A–C) The TNF-α, IL-1β, and IL-6 mRNA levels in macrophage. (D–F) The TNF-α, IL-1β, and IL-6 protein expression levels in macrophage. β-actin was used as a control. The macrophage was with or without H_2O_2 stimulating. SeD, incubated with selenomethionine 0 μM. SeN, incubated with selenomethionine 1.0 μM. SeS, incubated with selenomethionine 10 μM. Data are presented as the means ± SD. (n = 10 per group). *p < 0.01 indicates a significant difference from the SeS cells.

of ROS depends on various antioxidant enzymes [30]. Se plays an important role in the activity of various antioxidant enzymes [9]. GPx is one of the most important free radical trapping enzymes in the body. GPx was also the earliest known bioactive form of Se. GPx activity was markedly decreased with Se deficiency in the head kidney tissues [10]. CAT and SOD are also important for eliminating free radicals. CAT removes ROS from the body and reduces them to water [31]. SOD is the primary substance for scavenging free radicals in the biological body [32]. SOD can combat and block the damage caused by oxygen free radicals to cells and repair damaged cells at the same time. In the present study, CAT and SOD activities significantly decreased in the SeD SOD. MDA is the end product of oxidation when the ROS acts on lipids to produce the peroxidation reaction. MDA can cause proteins, nucleic acids, and other life macromolecules to crosslink, and it is cytotoxic [33]. MDA levels were markedly increased in the SeD group. With the increase in the Se concentration, MDA showed a decreasing trend. This

result further confirmed that Se deficiency induced the ROS increase.

It has been proved that there are more than 60 diseases caused by ROS. Excessive production of ROS may induce inflammation. The pro-inflammatory cytokines are indicators of inflammation and aggravated tissue damage. TNF-α is defined as an "early" cytokine [34]. IL-1β plays an important role in the regulation of the host immune responses [35]. In present study, it was found TNF-α, IL-1β, and IL-6 were appreciably increased in the SeD group. The results presented in this study show that Se deficiency and ROS induced inflammation. A previous study reported ROS can activate MAPKs signaling pathways by inducing secretion of inflammatory factors [36,37]. The MAPK pathway plays an important role in the regulation of inflammatory factors. The biological regulation is achieved by protein phosphorylation. The results presented in this study showed that phosphorylation of p38, JNK and ERK varied significantly. The phosphorylation was noticeably increased in the SeD group.

MicroRNA (micRNA) is a class of noncoding, small molecule, single-stranded RNA involved in immune defense, apoptosis, free radical injury, and other biological processes [15]. Se has shown effects on inflammation by regulating the expression of micRNA-146a and the MAPK pathway [23], and the ROS induced inflammation was related to micRNA-146a. To further reveal the molecular mechanisms of Se deficiency that induce an inflammatory response, micRNA-146a was analyzed. The expression of micRNA-146a significantly decreased in the SeD group, and a slight increase was found in the SeS group. This finding was consistent with previous research. micRNA-146a inhibits inflammation, so high levels of expression will reduce pro-inflammatory cytokine production [38].

To further confirm that ROS play a role in the inflammatory response induced by selenium deficiency and regulate inflammatory signals, macrophage cells from the head kidney were prepared and stimulated with H_2O_2. H_2O_2 is often used to simulate oxygen free radical damage in vitro [39]. The results displayed micRNA-146a significantly increased with the H_2O_2 stimulus. The increase was very weak in the SeD group. At the same time, p38, JNK and ERK phosphorylation was significantly increased, but this phosphorylation increase was significantly reduced in the SeS group. These results strongly support our findings in head kidney tissues. We further observed that pro-inflammatory cytokines were produced when inflammation was induced by ROS. TNF-α, IL-1β and IL-6 production were significant increased with H_2O_2 stimulus, but the increase was significantly inhibited with the Se increase. These results revealed ROS induced inflammation was due to the lack of Se.

In summary, this study showed that dietary Se deficiency induced excessive accumulation of ROS, resulting to inflammation in the head kidney of carp. At same time, Se effected the regulation of the expression of micRNA-146a and the MAPK pathway in the head kidney. The results indicated Se deficiency inhibits micRNA-146a to promote ROS-induced inflammation via regulating the MAPK pathway in the head kidney of carp. The present study reveals that supplementing the diet of carp with selenium is beneficial for growth and disease prevention.

Acknowledgments

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