The potential mediation of peroxiredoxin 6 in the activation of mitochondrion-dependent apoptosis during early postmortem of bovine muscle

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Introduction: Apoptosis is classified as a highly organized programmed cell death, which orchestrated by the caspase system belonging to the cysteine protease family. Caspase-3 is reported as the key effector molecule for apoptosis while the mitochondrial endogenous pathway mediated by caspase-9 is the main pathway for the activation of pro-caspase-3 in cells[1]. The hypoxia and ischemia of muscle cells directly caused by slaughter and bleeding of animals, which results in redox changes and increases of reactive oxygen species (ROS) that produce oxidative stress and accelerated apoptosis[2, 3].

Peroxiredoxin 6 (Prdx6) was recognized as an antioxidant enzyme that plays a role in protecting cells from oxidative stress, in other words, as scavenging reactive oxygen, Prdx6 can also repair membrane damage caused by oxidative stress and prevent cells from apoptosis[4]. However, the mechanism of Prdx6 on beef apoptosis has not been systematically reported. Therefore, in current study, beef underwent the treatment with NSC348884 which can inhibit the expression of Prdx6 to explore the effect of Prdx6 on mitochondrial pathway of apoptosis in postmortem beef from protein expression and apoptosis rate.

Materials and methods: The M. longissimus lumborum (LL) were immediately collected from three crossbred Simmental bulls in a commercial abattoir within 40 min after bleeding. The pH24 value were determined to avoid abnormal beef as the sample with pH24 5.4–5.8 was collected. Amount of 60 g meat from each LL was immersed in liquid nitrogen serving as 0 h samples while each loin was cut into 1*1*0.5cm3 pieces for incubation. Those beef cuts were randomly separated into four groups (incubated with or without inhibitors (52μM NSC348884, ratio of 1:1 (w/v)) for 5 time points (1, 6, 12, 24 and 36 h). After the incubation, each sample was incubated individually with or without H2O2 for 30 min. Thus, four treatments were included (without inhibitor & with H2O2, marked as CH group, and inhibited & with H2O2, marked as NH group, and without inhibitor or H2O2 as CC group, and inhibited & without H2O2, marked as NC group). Then, 4% paraformaldehyde and liquid nitrogen was used to keep samples for Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) and western blotting analysis, respectively. Apoptotic nuclei counts and protein expression of samples were examined. The MIXED procedure of SAS Version 9.0 was used with treatment, storage time and their interaction as fixed factors and carcass as a random factor. Least squares means were separated using the PDIFF option and were considered significant at $P < 0.05$.

Results: The expression of Prdx6 in the control groups (CC group and CH group) were significantly higher compared with those in the NSC348884 treatment groups (NC group and NH group). To evaluate the effect of protein Prdx6 on the apoptosis during postmortem, we further examined the expression of caspase-3 and caspase-9 in the four groups. The results clearly suggest the inhibition of Prdx6 accelerated the activation of caspase-3 and caspase-9 during early post-mortem ($P < 0.05$). In addition, with the extension of post-mortem time, the number of apoptotic nuclei exhibited an increase between 1 and 36 h postmortem, and there were almost no apoptotic nuclei after incubation for 1 h. Furthermore, at the same incubation time, compare with CC group as well as CH group, the number of apoptotic nuclei in NC and NH groups increased obviously, and apoptotic nucleus exhibited the most richness in NH group. These results indicated that the inhibition of Prdx6 increase the apoptosis process in beef, as oxidative stress could increase apoptosis to a certain extent. In this study, the degree of nuclear apoptosis was consistent with the degradation degree trend of caspases.

Conclusions: The results suggested that the Prdx6 level can be depressed by NSC348884, while the inhibition of Prdx6 significantly increased the activation of caspase-3 and caspase-9, which demonstrated that Prdx6 contributed to the inhibition of apoptotic mitochondrial pathway. Meanwhile, the inhibition of Prdx6 and the oxidative stress treatment obviously promoted the apoptosis rate, that means, Prdx6 plays a role in combating oxidative stress and preventing apoptosis.

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References:

[1] HOU Q, LIU R, TIAN X, et al. Involvement of protein S-nitrosylation in regulating beef apoptosis during postmortem aging[J]. Food Chemistry, 2020, 326. 126975. DOI:10.1016/j.foodchem.2020.126975.
[2] WANG L L, YU Q L, HAN L, et al. Study on the effect of reactive oxygen species-mediated oxidative stress on the activation of mitochondrial apoptosis and the tenderness of yak meat[J]. 2017, 244.394.
[3] CHEN C, ZHANG J, GUO Z, et al. Effect of oxidative stress on AIF- mediated apoptosis and bovine muscle tenderness during postmortem aging[J]. Journal of Food Science, 2019, 85. DOI:10.1111/1750-3841.14969.
[4] FISHER A B. Peroxiredoxin 6 in the Repair of Peroxidized Cell Membranes and Cell Signaling[J]. Archives of biochemistry and biophysics, 2017, 617. 68-83. DOI:10.1016/j.abb.2016.12.003.