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TISSUE-SPECIFIC RESPONSES OF PROTEIN OXIDATIVE DESTRUCTION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) EXPOSED TO DISINFECTANT "CIP" FORMULATED WITH PERACETIC ACID AND HYDROGEN PEROXIDE

The aim of this study was to evaluate the impacts of disinfection processes with disinfectant "CIP" *based on 15% peracetic acid (PAA) and 20% H_2O_2 on the aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle, gill, hepatic and cardiac tissues of juvenile rainbow trout (Oncorhynchus mykiss). In the disinfectant exposure, fish were bathed with Disinfectant "CIP" solution in final concentration 16 mL per m^3 (Group II). Fish were bathed for 20 min and repeated three times every 3 days. Bathing with Disinfectant "CIP" were taken in the morning before fish feeding. Control fish (Group I) were handled at same manner as Group II but without Disinfectant "CIP" treatment. Two days after the last bathing, twenty two individuals from each group were sampled. Fish were not anesthetized before tissue sampling. Findings of this study showed that protein damage in gill tissue is decreased in "CIP"-exposed group when compared with control group. Carbonyl derivatives as biomarkers of protein damage in the muscle tissue showed a downward trend after disinfectant treatment. Similarly, oxidative modification of protein also showed a downward trend after disinfection treatment in the hepatic tissue. In this study, no significant changes in carbonyl contents as oxidative damage of proteins of hepatic and cardiac tissues of "CIP"-treated group compared with control group was observed. Our studies indicated that "CIP"-Disinfectant* in final concentration 16 mL per m^3 *could at least partly attenuate protein damage and can be used for prophylactic disinfecting treatment of rainbow trout.*

Key words: *peracetic acid, hydrogen peroxide, disinfection, oxidatively modified proteins, aldehydic and ketonic derivatives, muscle, gill, liver, heart.*

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ТКАНЕСПЕЦИФИЧЕСКИЕ ИЗМЕНЕНИЯ В СОДЕРЖАНИИ ОКИСЛИТЕЛЬНО-МОДИФИЦИРОВАННЫХ БЕЛКОВ В РАДУЖНОЙ ФОРЕЛИ (*ONCORHYNCHUS MYKISS* WALBAUM), ДЕЗИНФИЦИРОВАННОЙ «CIP»-ДЕЗИНФЕКТАНТОМ НА ОСНОВЕ ПЕРУКСУСНОЙ КИСЛОТЫ И ПЕРЕКИСИ ВОДОРОДА

Цель данного исследования – оценить воздействие процессов дезинфекции дезинфицирующим средством «CIP» на основе 15 % перуксусной кислоты и 20 % H_2O_2 на содержание альдегидных и кетоновых производных окислительно-модифицированных белков в мышцах, жабрах, печеночной и сердечной тканях радужной форели (Oncorhynchus mykiss). Воздействие дезинфицирующего средства «CIP» в конечной концентрации 16 мл/м^3 состояло в купании рыб в этом растворе в течение 20 мин (группа II). Процедуру повторяли три раза через каждые 3 дня. Контрольную группу рыб (группа I) обрабатывали водой без дезинфектантов так же, как и опытную группу. Через два дня после последнего купания были отобраны двадцать две рыбы из каждой группы. Результаты этого исследования показали, что содержание карбонильных производных окислительно-модифицированных белков в жаберной ткани снижается в дезинфицированной группе рыб по сравнению с контрольной. Кетоновые производные как биомаркеры повреждения белка в мышечной ткани показали также тенденцию к снижению после дезинфицирующих мероприятий. Аналогичным образом

окислительная модификация белка также продемонстрировала тенденцию к снижению после дезинфекционной обработки в ткани печени. В этом исследовании не было обнаружено существенных изменений содержания карбонилов в печеночной и сердечной тканях после обработки дезинфектантом «CIP». Наши исследования показали, что дезинфицирующее средство «CIP» в конечной концентрации 16 мл/м^3 может, по меньшей мере, частично ослабить повреждение белка в тканях и может быть использовано для профилактических дезинфекцирующих мероприятий у радужной форели.

Ключевые слова: *перуксусная кислота, перекись водорода, дезинфекция, окислительно-модифицированные белки, альдегидные и кетоновые производные, мышцы, жабры, печень, сердце.*

Introduction

Peracetic acid (PAA) is a strong disinfectant with a wide spectrum of antimicrobial activity. PAA is a strong oxidizing disinfectant, which is currently sold in several commercial compounds. Strong PAA solutions have to be handled with care but the PAA (CH_3CO_3H) itself breaks down in water into acetic acid ($C_2H_4O_2$) and hydrogen peroxide (H_2O_2) and further into water and oxygen, which all are rather harmless compounds [10]. The mechanism of the toxicity might be that PAA and H_2O_2 are exogenous sources of reactive oxygen species (ROS). They can pass the cell membrane and increase the endogenous ROS concentration [12]. Hydrogen peroxide can transport across membranes by free diffusion and is scavenged by antioxidant enzymes, i.e. catalase and glutathione peroxidase under normal conditions [2]. Excess hydrogen peroxide can react with free Cu and Fe in the cytosol and form hydroxyl radicals leading to damage in macromolecules [4].

Useful effects of PAA application in aquaculture are very well documented [5, 9, 14-21]. Aquaculture-related research with PAA products includes *in vitro* assessments, where promising disinfection action has been documented [14-21]. The demonstrated *in vitro* efficacy of the PAA-based product suggests its great potential especially to control *Ichthyophthirius multifiliis* infections in commercial aquacultural systems [17]. PAA concentrations of 0.3 ppm were able to kill all theronts of *I. multifiliis* in 120 min. Meinelt and co-workers (2007) recommend an interval-application of 0.3 to 0.5 ppm PAA for 30 to 150 min and should be prolonged for two life cycles of the parasite [15]. Biotic parameters as e. g. fish species, and age as well as abiotic parameters as e. g. temperature, pH and organic load of the water could possibly influence the efficiency of the PAA application and should therefore be taken into account while picking the dosage and length of the PAA exposure [15]. Straus and Meinelt (2009) have determined the acute toxicity of two products containing 4.5% and 40% PAA to *I. multifiliis* theronts from two geographically separate isolates [18]. Theronts were exposed to concentrations of PAA in 96-well plates containing groundwater at 23 °C. Acute toxicity was observed over a 4-h period. No significant difference in the median lethal concentration (LC_{50}) estimates was evident between the two isolates at 4 h with the 4.5% PAA product (0.146 versus 0.108 mg/l PAA), while there was a statistical difference between the 4 h LC_{50} with the 40% PAA product (0.274 versus 0.158 mg/l PAA) [18].

Straus and co-workers (2012) determined the effectiveness of PAA for fungus control on channel catfish (*Ictalurus punctatus,* Rafinesque) eggs. The study consisted of five PAA concentrations (2.5, 5, 10, 15 and 20 mg·L^{-1}) and an untreated control in a flow-through system. Eggs were treated twice daily until the embryos developed eyes. When hatching was complete for all viable eggs, fry were counted to determine the percent survival in each treatment. Fungal growth was severe in the untreated controls resulting in 11% survival. Treatments of 2.5, 5 and 10 mg·L^{-1} PAA were significantly different from the controls ($P<0.05$). The highest percent survival of hatched fry was with 5 mg·L^{-1} PAA administered twice daily; the 2.5 mg·L^{-1} PAA treatment had slightly less survival, but gives a higher margin of safety in case of treatment error. Very little fungus was present in treatments receiving 2.5 mg·L^{-1} PAA or higher, and concentrations of 15 and 20 mg·L^{-1} PAA were toxic to the eggs. The mean survivals in the 0, 2.5, 5, 10, 15 and 20 mg·L^{-1} PAA treatments were 11%, 60%, 63%, 62%, 32% and 0%, respectively [20].

Therefore, the aim of this study was to evaluate the impacts of disinfection processes with CIP-Disinfectant based on 15% PAA and 20% H_2O_2 on the aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle, gill, hepatic and cardiac tissues of juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum).

Materials and methods

Fish. Juveniles of rainbow trout at the age of 0+ (in the first year of life) came from spawning in Department of Salmonid Research, Inland Fisheries Institute (Rutki, Zukowo, Poland). Fish were fed daily of feed with using of tape feeders. Feed daily dose was calculated according to the applicable feed table, depending on the water temperature. Fish were starved one day prior to experiment. Forty four clinically healthy rainbow trout (*O. mykiss*) with a mean body mass of 45.8±1.2 g were used in the experiments. Experiments were performed at a water temperature of 16±2 °C and the pH was 7.5-7.6. The dissolved oxygen level was about 12 ppm with additional oxygen supply. Every morning were measured temperature and the oxygen content in the water, using thermometer and oxygen meter, respectively. All enzymatic assays were carried out at Department of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University in Slupsk (Poland).

The fish were divided into two groups and held in 250-L square tanks (70 fish per tank). As disinfecting agent was used Disinfectant "CIP" solution based on 15% PAA and 20% H_2O_2 (firm "Biochem-Art", Gdansk, Poland). It was admitted to market by the license No. 0508/04 of Minister of Health in Poland (27.01.2004) as biocidal product named Disinfectant "CIP" [8]. In the disinfectant exposure, fish were bathed with Disinfectant "CIP" solution in final concentration 16 mL per m^3 (Group II). Fish were bathed for 20 min and repeated three times every 3 days. Bathing with Disinfectant "CIP" were taken in the morning before fish feeding. Control fish (Group I) were handled at same manner as Group II but without Disinfectant "CIP" treatment. Two days after the last bathing, twenty two individuals from each group were sampled. Fish were not anesthetized before tissue sampling.

Isolation of tissues. Muscle, gill, hepatic and cardiac tissues were removed from trout after decapitation. One trout was used for each homogenate preparation. Briefly, tissues were excised, weighted and washed in ice-cold buffer. The minced tissue was rinsed clear of blood with cold isolation buffer and homogenized in a glass homogenizing vessel with a motor-driven pestle on ice. The isolation buffer contained 100 mM tris-HCl; pH of 7.2 was adjusted with HCl.

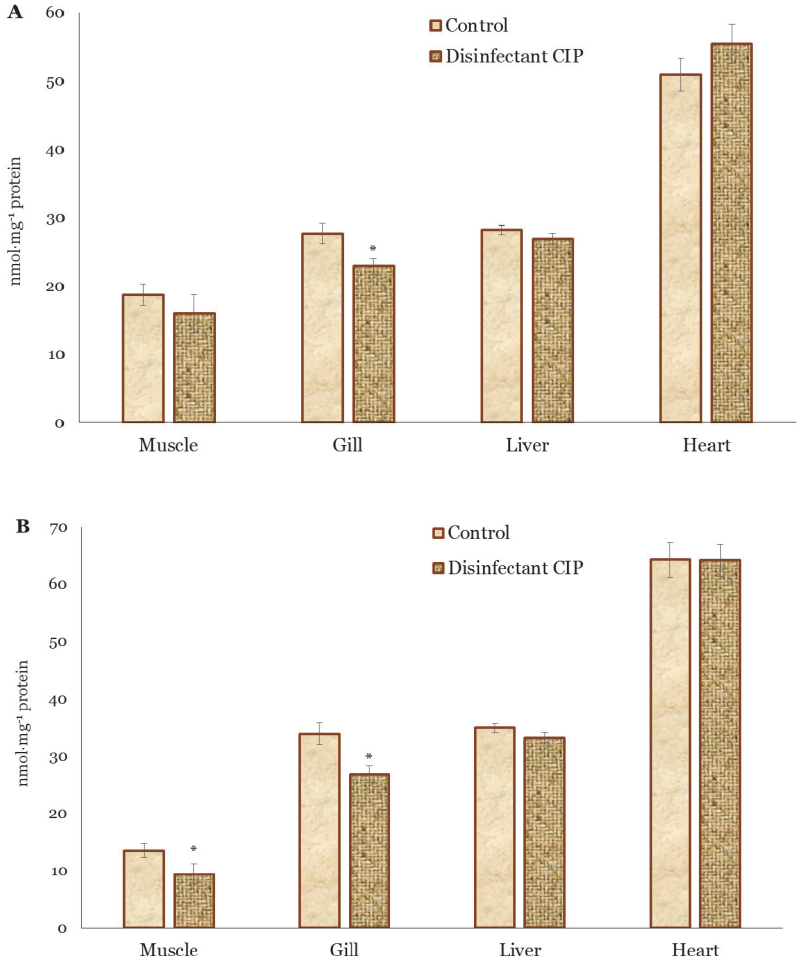
Biochemical assays. All enzymatic assays were carried out at 25±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany). The enzymatic reactions were started by adding the homogenate suspension. Each sample was analyzed in triplicate. The protein concentration in each sample was determined according to Bradford (1976) using bovine serum albumin as a standard [3]. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-Dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) [11] and as modified by Dubinina and co-workers (1995) [7]. DNPH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}) and expressed in nmol per mg of tissue protein.

Statistical analysis. The obtained results were analyzed statistically using the Statistica 8.0 software package (StatSoft, Krakow, Poland). Results are expressed as mean ± S.E.M. Kolmogorov-Smirnov test was applied to evaluate normality of the data. As most of the distributions deviated from the normal distribution, non-parametric tests were used for further analyses. To assess the differences between the studied group and untreated control, the non-parametric Mann-Whitney *U*-test was used, and P-values of less than 0.05 were considered as significant [22].

Results and discussion

Oxidized proteins are increased with oxidative stress and in some pathological conditions [13]. The most protein modification caused by oxidative stress is the carbonyl groups; the most sensitive amino acids are arginine, lysine, proline, threonine, and glutamic acid. Reactive protein carbonyls reflect the degree of oxidative damage and serve as a biomarker for oxidative stress [13]. For detection of reactive protein carbonyl groups, several methods are available, including DNPH assay [11]. Thus, levels of aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle, gill, hepatic and cardiac tissues of juvenile rainbow trout of unhandled and Disinfectant "CIP"-exposed group were presented in Fig. 1.

Carbonyls contents as biomarkers of oxidative stress showed non-significant changes between unhandled and Disinfectant "CIP"-exposed group except muscle and gill tissues (figure). Ketonic derivatives of protein carbonyls content in these tissues of "CIP"-exposed trout was lowered by 31% (p=0.003) and by 21% (p=0.003) respectively compared to control group (Fig. 1B). Aldehydic derivatives of oxidatively modified proteins in gill tissue of Disinfectant "CIP"-exposed trout was also decreased by 17.5% (p=0.007) (Fig. 1A).



Aldehydic (A) and ketonic derivatives (B) of oxidatively modified proteins in the muscle, gill, hepatic and cardiac tissues of juvenile rainbow trout of control and "CIP"-exposed group.
Data are represented as mean ± S.E.M. (n=11). * – the significant change was shown as $p<0.05$ when compared values of control and disinfected groups

The aim of this study was to evaluate the effect of Disinfectant "CIP" based on PAA and H_2O_2 on aldehydic and ketonic derivatives of oxidatively modified proteins in muscle, gill, hepatic and

cardiac tissues of rainbow trout. Findings of this study showed that protein damage in gill tissue is decreased in “CIP”-exposed group when compared with control group (figure). Carbonyl derivatives as biomarkers of protein damage in the muscle tissue showed a downward trend after disinfectant treatment. Similarly, oxidative modification of protein also showed a downward trend after disinfection treatment in the hepatic tissue. In this study, no significant changes in carbonyl contents as oxidative damage of proteins of hepatic and cardiac tissues of “CIP”-treated group compared with control group was observed (figure).

The most likely explanation is a tissue-specific difference in the intracellular levels of oxidized proteins, reflecting the balance between the rate of protein oxidation and the rate of oxidized protein degradation [13]. This balance is a function of factors leading to the generation of ROS. Various physiological and environmental processes may lead to the formation of ROS and be factors in determining the concentrations and/or activities of the proteases that degrade oxidatively damaged proteins [1]. From *in vitro* studies it is evident that the 20S proteasome complex actively recognizes and degrades oxidized proteins. Furthermore, relatively mild oxidative stress rapidly (but reversibly) inactivates both the ubiquitin activating/conjugating system and 26S proteasome activity in intact cells, but does not affect 20S proteasome activity. Since mild oxidative stress actually increases proteasome-dependent proteolysis (of oxidized protein substrates) the 20S 'core' proteasome complex would appear to be responsible [6]. Such degradation is also dependent upon numerous variables, including the concentrations of proteases that preferentially degrade oxidized proteins, and upon cellular components, such as metal ions, inhibitors, activators, and regulatory proteins, that affect their proteolytic activities. For example, oxidized forms of some proteins, for example, cross-linked proteins and proteins modified by glycation or by lipid peroxidation products, are resistant to proteolysis and could lead to production of protease inhibitors that hinder degradation of the oxidized forms [13].

Conclusions

Aldehydic and ketonic derivatives of oxidatively modified proteins were affected after exposure of rainbow trout to Disinfectant “CIP”. However, the observed effects were dependent on fish tissues. Exposure to Disinfectant “CIP” affected ketonic derivatives only in the muscle and gill tissues, while changes in aldehydic derivatives of OMP were observed in gill tissue. Tissues were able to protect themselves against the possible potential oxidative damage induced by Disinfectant “CIP”. Moreover, no significant alterations in aldehydic and ketonic derivatives of oxidatively modified proteins in hepatic and cardiac tissues of disinfected trout were observed.

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