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Тканеспецифические ответные реакции окисления
белков у хариуса (*Thymallus thymallus* L.)
дезинфицированного хлорамином-Т

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Tissue-specific response of protein oxidation in the grayling
(*Thymallus thymallus* L.) disinfected by chloramine-T

Хлорамин-Т широко используется как дезинфицирующее средство для лечения жаберных заболеваний пресноводных рыб, а в последнее время стали обращать внимание также на его использование и в морской воде. Тем не менее, несмотря на широкое использование хлорамина-Т, существует мало исследований его токсичности для рыб. Целью настоящего исследования было изучение влияния хлорамина-Т на уровень альдегидных и кетонных производных окислительно модифицированных белков в мышечной ткани, жабрах, печени и сердце хариуса (*Thymallus thymallus* L.). Наши результаты показали, что дезинфекция хлорамином-Т увеличивает содержание альдегидных и кетонных производных окислительно модифицированных белков в ткани печени. Значительное снижение альдегидных и кетонных производных наблюдалось в сердечной ткани хариуса. Наши исследования показали, что хлорамин-Т в дозе 9 мг на литр может быть использован для профилактической дезинфекции хариуса. Производные окислительной модификации белков могут быть эффективно использованы в качестве потенциальных биомаркеров токсичности хлорамина-Т в аквакультуре. Тем не менее, нужны более подробные исследования по использованию этих конкретных биомаркеров для мониторинга дезинфицирующих мероприятий в аквакультуре.

Ключевые слова: хлорамин-Т, окислительный стресс, хариус (*Thymallus thymallus* L.)

Chloramine has been used as disinfectant for over a hundred years, but in the last decade it has become increasingly popular. The main reason for using it as an alternative for chlorine is because it generates fewer byproducts. The aim of the present study was to examine the effects of chloramine-T exposure on the levels of aldehydic and ketonic derivatives of oxidatively modified proteins products in muscle tissue, gills, liver, and heart of grayling (*Thymallus thymallus* L.). In the disinfectant exposure, grayling were exposed to chloramine-T in final concentration 9 mg per L. Fish were bathed for 20 min and repeated three times every 3 days. Our results showed that chloramine-T bathing markedly increase aldehydic and ketonic derivatives of oxidative protein in hepatic tissue. Significantly decrease of aldehydic and ketonic derivatives in cardiac tissue was observed. Aldehydic and ketonic derivatives of oxidatively modified proteins could be effectively used as potential biomarkers of chloramine-T toxicity to the fish in the warning signal for pharmaceutical exposure to aquatic organisms. However, more detailed studies on using of these specific biomarkers to monitor the disinfectant treatment in aquaculture are needed.

Key words: chloramine-T, oxidative stress, grayling (*Thymallus thymallus* L.)

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Introduction

The general principles pertaining to disinfection of aquaculture establishments involve the application of chemical treatments in sufficient concentrations, and for sufficient periods, to kill all pathogenic organisms that would otherwise gain access to surrounding water systems. There are many chemical disinfectants currently utilized in aquaculture. Common ones include halogens such as chlorine and iodine, quaternary ammonium compounds, alcohols such as isopropanol and ethanol, phenolic compounds such as cresol, oxidizing agents such as peroxide and peracetic acid, and alkylating agents such as formalin, glutaraldehyde, and ethylene oxide [8]. Chloramine has been used as disinfectant for over a hundred years, but in the last decade it has become increasingly popular. The main reason for using it as an alternative for chlorine is because it generates fewer byproducts [4]. It is commonly used as therapeutic agent for the treatment of bacterial and parasitic gill and skin diseases in the intensive aquaculture of salmonids [5,10,11].

Chloramine-T is an organic N-chloramine. Chloramine-T is an exception to the organic chloramines because of its considerable value as a disinfectant and sanitizer. Organic chloramines in general are thought to be considerably less toxic to aquatic life than the inorganic chloramines monochloramine (NH_2Cl), dichloramine (NHCl_2), and trichloramine (NCl_3). Inorganic chloramines usually exist as monochloramine in aqueous solutions. Both aqueous free chlorine ($\text{HOCl} + \text{OCl}^-$) and the inorganic chloramines are extremely toxic to fish and other aquatic life, to the point where concentrations of $<10 \mu\text{g/L}$ (total of free chlorine plus inorganic chloramines) are potentially of concern [13]. On the other hand, disinfectants cause oxidative stress that may stimulate the generation of reactive oxygen species (ROS), and subsequently the alteration in antioxidant systems of exposed organisms. The involvement of oxidative stress and chlorination of the cellular materials has been suggested as a mechanism for chloramine-Toxicity, but the mechanism of this disinfectant selective toxicity in tissues is still unclear. Cells exposed to low concentrations of cell-permeable chloramines show increased membrane permeability and potassium loss. These oxidants have also been shown to inhibit oxygen uptake and glucose metabolism and cause loss of ATP and NAD, activate MAP kinase pathways and initiate growth arrest, influence NF κ B-dependent cytokine and adhesion molecule expression, affect NO metabolism, and induce ROS generation and apoptosis [14].

The aim of the present study was to examine the effects of chloramine-T exposure on the levels of aldehydic and ketonic derivatives of oxidatively modified proteins in muscle tissue, gills, liver, and heart of grayling (*Thymallus thymallus* L.).

Materials and methods

Experimental Fish. Twenty clinically healthy grayling, 1+ year of age, were used in the experiments. An average body weight of fish was 28.6 ± 0.18 g. The study was carried out in a Department of Salmonid Research, Inland Fisheries Institute near the village of Żukowo, Poland. Experiments were performed at a water temperature of $16 \pm 2^\circ\text{C}$ and the pH was 7.5. All biochemical assays were carried out at Department of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University (Słupsk, Poland). The fish were divided into two groups and held in 250-L square tanks (70 fish per tank) supplied with the same water as during the acclimation period (2 days). On alternate days, the water supply to each tank was stopped. In the disinfectant exposure, grayling were exposed to chloramine-T in final concentration 9 mg per L. Control groups of grayling were handled in the same way with chloramine-T treatment groups. Fish were bathed for 20 min and repeated three times every 3 days. Two days after the last bathing fish were sampled.

Tissues isolation. Muscle tissue, gills, livers, and heart were removed from grayling after decapitation. One grayling was used for each homogenate preparation. Tissue sample were homogenized in ice-cold buffer (100 mM Tris-HCl, pH 7.2) using a glass homogenizer to a yield a 10% homogenate. Homogenates were centrifuged at 3,000g for 15 min at 4°C . Protein contents were determined using the method of Bradford (1976) with bovine serum albumin as a standard [1]. Absorbance was recorded at 595 nm. All enzymatic assays were carried out at $22 \pm 0.5^\circ\text{C}$ using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate.

Carbonyl derivatives of oxidatively modified protein (OMP) assay. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of aminoacid reaction with 2,4-dinitrophenyl hydrazine as described by Levine et al. (1990) [6] and as modified by Dubinina et al. (1995) [3]. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP370) and 430 nm (ketonic derivatives, OMP430) and expressed in nmol per mg of tissue protein.

Statistical analysis. Data were checked for assumptions of normality using the Kolmogorov–Smirnov one-sample test and Lilliefors tests ($p > 0.05$). In order to find significant differences (significance level, $p < 0.05$) between control and chloramine-T exposed groups, Mann-Whitney U test was applied to the data [17]. Differences were considered significant at $p < 0.05$. All statistical analysis was performed by STATISTICA 10.0 software (StatSoft, Poland).

Results

Our results showed that chloramine-T bathing markedly increase aldehydic and ketonic derivatives of oxidative protein in hepatic tissue by 10.6% ($p = 0.023$) and by 8.4% ($p = 0.049$), respectively (Fig. 1). Significantly decrease of aldehydic and ketonic derivatives in cardiac tissue was observed (by 11.2%, $p = 0.008$ and by 10.9%, $p = 0.041$, respectively) (Fig. 1).

* the significant change was shown as $p < 0.05$ when compared to control group values
Each value represents the mean $\pm$ S.E.M.

Fig. 1. Aldehydic and ketonic derivatives of oxidative protein modification in the muscles, gills, liver, and heart of grayling exposed to chloramine-T bathing

Discussion and conclusions

Our results showed that chloramine-T bathing markedly increase aldehydic and ketonic derivatives of oxidative protein in hepatic tissue. Significantly decrease of aldehydic and ketonic derivatives in cardiac tissue was observed. Aldehydic and ketonic derivatives of oxidatively modified proteins could be effectively used as potential biomarkers of chloramine-T toxicity to the fish in the warning signal for pharmaceutical exposure to aquatic organisms.

In toxic chloramine-treated animals, the histological changes of fish tissue, such as structure damage, necrosis, alterations and hemorrhage, had been observed [11]. Powell et al. (1994) have shown that repeated intermittent exposures to a therapeutic dose of chloramine-T (9 mg per L) to adult trout resulted in a mucous cell hyperplasia

that was partially compensated for by an elevated ventilation rate and a thinning of the lamellar epithelium. They also suggest that although there was a mucous cell hyperplasia in response to repeated chloramine-T exposure, the thinning of the lamellar epithelium was sufficient to offset any diffusive limitations, thus ensuring that gas exchange was not adversely affected [11]. Powell et al. (1994) confirmed, that chloramine-T can be used for repeated prophylactic treatment of adult trout with no apparent detrimental effects to gas exchange or metabolic rate [11].

In our previously study, to clarify the mechanism of hepatotoxicity caused by chloramine-T bathing, the oxidative stress, biochemical and enzymological biomarkers were compared among rainbow trout, *Oncorhynchus mykiss* (Walbaum), brown trout, *Salmo trutta m. fario* (L.), and grayling, *Thymallus thymallus* (L.). Fish were exposed to chloramine-T in final concentration 9 g per m³ for 20 min and repeated three times every 3 days. Control un-treated groups of fish were handled in the same way with chloramine-T treatment groups. The treatment of chloramine-T is varying from one species to another species of fish. Chloramine-T bathing markedly decrease level of carbonyl derivatives of oxidative protein, and aminotransferases activities only in liver of rainbow trout, and their elevation is a compensatory mechanism to impaired metabolism. No significant changes were found in oxidative stress biomarkers between control and chloramine-treated brown trout. For grayling, chloramine-T exposure caused significantly elevation in the levels of severe oxidative stress biomarkers. Increased carbonyl derivatives of oxidative protein could modify aminotransferases and LDH activities, lactate and pyruvate levels principally causing increased enzymes activity due to oxidative stress in the liver of chloramine-exposed fish. Our studies indicated that chloramine-T in dose 9 g per m³ could at least partly attenuate oxidative stress and can be used for prophylactic treatment to rainbow and brown trout [16].

Gills are potentially useful monitor organs to reflect the health of aquatic organisms and are in close contact with the water, which makes them significant targets for waterborne pollutants [2]. Liver is the primary organ site for xenobiotic metabolism [7]. In most cases, the metabolic process is accomplished without injury to the liver itself, whereas many xenobiotic compounds are toxic that can cause liver injury [9]. Accumulating evidence has shown that chloramine-T causes oxidative stress by inducing the generation of reactive oxygen species (ROS) [15]. Sakuma et al. (2009) found that monochloramine could increase ROS generation in the cytoplasm of rat primary hepatocyte cultures [12]. When incubated with the partially purified cytosolic fraction from rat liver, monochloramine (2.5-20 microM) dose-dependently enhanced xanthine oxidase activity concomitant with a decrease in xanthine dehydrogenase activity, implying that monochloramine can convert xanthine dehydrogenase into the ROS producing form xanthine oxidase. It was found that monochloramine could increase ROS generation in the cytoplasm of rat primary hepatocyte cultures, and that this increase might be reversed by an xanthine oxidase inhibitor, allopurinol. These results suggest that monochloramine has the potential to convert xanthine dehydrogenase into xanthine oxidase in the liver, which in turn may induce the ROS generation in this region [12].

HOCl and the model N-chloramine are able to chlorinate cellular genetic material, which may play a role in the development of various inflammatory cancers [15]. They have examined the ability of various N-chloramines to form chlorinated base products on nucleosides, nucleotides, DNA, and in cellular systems [15]. Stanley et al. (2010) examined the ability of HOCl and various N-chloramines to form chlorinated base products on nucleosides, nucleotides, DNA, and in cellular systems. Experiments were performed with N-chloramines formed on N α -acetyl-histidine (His-C), N α -acetyl-lysine (Lys-C), glycine (Gly-C), taurine (Tau-C), and ammonia (Mono-C). Treatment of DNA and related materials with HOCl and N α -acetyl-histidine resulted in the formation of 5-chloro-2'-deoxycytidine, 8-chloro-2'-deoxyadenosine and 8-chloro-2'-deoxyguanosine. Cellular RNA was also a target for HOCl and His-C, with evidence for the formation of 5-chloro-cytidine. HOCl and the model N-chloramine, His-C, are able to chlorinate cellular genetic material, which may play a role in the development of various inflammatory cancers [15].

These results suggested that chloramine-T in dose 9 mg per L is safe and could decrease the oxidative stress in cardiac tissue of grayling (Fig. 1). Our results are also consistent with the view that the liver has high resistance to oxidative stress. Consequently, if some measure of chloramine-T exposure occurs, the liver might readily handle the resulting oxidative stress. Our studies indicated that chloramine-T in dose 9 mg per L could at least partly activate oxidative stress in hepatic tissue grayling. No significant changes in protein damage levels in the muscle and gill tissue were observed and can be used for prophylactic treatment of grayling (Fig. 1). Aldehydic and ketonic derivatives of oxidatively modified proteins could be effectively used as potential biomarkers of chloramine-T toxicity to the fish in the warning signal for pharmaceutical exposure to aquatic organisms. However, more detailed studies on using of these specific biomarkers to monitor the disinfectant treatment in aquaculture are needed.

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