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BIOCHEMICAL CHANGES IN THE MUSCLE TISSUE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) DISINFECTED BY CHLORAMINE-T

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Abstract

Chloramine-T is a widely used disinfectant for the treatment of gill diseases of fish in freshwater, and more recently attention has turned to its use in seawater. However, despite the wide use of chloramine-T, few studies have examined its toxicity to fish. Therefore, the aim of the current study was to examine the effects of disinfection by Chloramine-T on the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) using oxidative stress biomarkers (levels of 2-thiobarbituric acid reactive substances and derivatives of oxidatively modified proteins) and biochemical enzymes' activity (alanine- and aspartate aminotransferases (ALT and AST), lactate dehydrogenase (LDH)) to observe the its toxic effects. The endpoints obtained from this study will be useful to monitor the effects of disinfectant bathing with Chloramine-T for this species of fish. In the disinfectant group, rainbow trout (n = 11) were exposed to Chloramine-T in final concentration of 9 mg per L. Control group of trout (n = 11) was handled with water from basin in the same way as Chloramine-T exposed group. Fish were bathed with Chloramine-T for 20 min and repeated three times every 3 days. Two days after the last bathing fish were sampled to study. Our results showed that Chloramine-T bathing caused the decrease of the lipid peroxidation as well as ALT and AST activity and significant decrease of LDH activity (by 339%, p = 0.017) compared to controls. Chloramine-T markedly affects on lactate and pyruvate metabolism and resulted to decrease of LDH activity. Correlative analysis revealed that the lipid peroxidation level is correlated with ALT and AST activity in the muscle tissue of unhandled control group. In the muscle tissue of trout disinfected by Chloramine-T, LDH activity is correlated positively with ALT and AST activity. Thus, the skeletal muscles of fish play an important role in the processing of lactate through the gluconeogenic and glycogenic pathways including a greater potential for biosynthesis. Our studies indicated that Chloramine-T in dose of 9 mg per L could at least partly attenuate oxidative stress and

can be used for prophylactic disinfecting treatment of rainbow trout. Oxidative stress and biochemical alterations 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 disinfection, rainbow trout (*Oncorhynchus mykiss* Walbaum), muscle tissue, lipid peroxidation, aminotransferases, lactate dehydrogenase

INTRODUCTION

The use of pharmaceutical substances is rather limited in fish compared to mammalian therapeutics. It is basically restricted to anaesthetic agents and anti-infective agents for parasitic and microbial diseases. The anti-infective agents are used for controlling diseases and the choice of drug depends on efficacy, ease of application, human safety, target animal safety including stress to the fish, environmental impact, regulatory approval, costs, and implications for marketing the fish (Burka et al. 1997). In fish aquaculture, disinfectants are used against bacterial and protozoal infections. These compounds cause oxidative stress that may stimulate the generation of reactive oxygen species, and subsequently the alteration in antioxidant systems of exposed organisms (Stara et al. 2014).

Chloramine-T, as an anti-microbial agent, has had widespread use in a broad range of practices, including medical, dental, veterinary, food processing, and agricultural. As a disinfectant, it is used to disinfect surfaces and instruments. Chloramine-T has a low degree of cytotoxicity and has been used in direct contact with tissues. It is easy to use and effective against many bacteria (both Gram-negative and -positive), viruses (enveloped and naked), fungi, algae, yeast, and parasites (Toxicological Summary... 2002).

Chloramine-T, a widely used chemotherapeutic and chemoprophylactic treatment for gill diseases in the freshwater aquaculture industry (Thorburn and Moccia 1993) was found to increase freshwater bathing efficacy and reduced amoeba survival (Powell and Clark 2003). Other studies also suggest that Chloramine-T in seawater is as effective in seawater as in fresh water (Harris et al. 2004, 2005). Chloramine-T has been widely used in the treatment of gill diseases in the freshwater aquaculture industry (Thorburn and Moccia 1993). Studies of Powell and Clark (2003) have shown that Chloramine-T is effective at killing Neoparamoeba spp. and its addition to seawater can produce efficacy levels similar to that found in freshwater treatment (Harris et al. 2004).

Sanchez and co-workers (1998) concluded that although Chloramine-T and formalin may continue to be useful in the aquaculture industry they cause potentially harmful alterations to fish skin. Juvenile rainbow trout were exposed to therapeutic concentrations of formalin or Chloramine-T to assess the effects of these chemicals on the morphology of the piscine epidermis and its mucous coat. Repeated treatment, once weekly for 4 weeks, with either chemical did not affect the mucous coat of the epithelium or the degree of folding of the basal lamina. However, treated fish had increased numbers of highly dense vesicles within the apical portions of epithelial

cells. The epidermal mucous cells of chloramine-T-treated fish were significantly smaller than in controls. This effect was not noted in formalin-treated fish. Treatment with either chemical resulted in a significantly thinned epidermis (Sanchez et al. 1998).

The toxicity of Chloramine-T (on the basis of several different endpoints) has been examined in a variety of fish species by several authors (Bootsma 1973, Cross and Hursey 1973, Bills et al. 1988a, b, 1993, Powell and Perry 1996, Environmental Assessment... 2007). Of the species tested, channel catfish, rainbow trout, and striped bass were similarly sensitive when tested in soft acidic water (Bills et al. 1988a, b, 1993). Chloramine-T 96-h LC_{50} values were 1.8 mg per L for channel catfish, 1.9 mg per L for rainbow trout, and 2.8 mg per L for striped bass (pH = 6.5). The 96-h LC_{50} values in waters of pH 7.5 for channel catfish, rainbow trout, striped bass, and fathead minnow, and in water of pH 7.7 for harlequin fish were 3.8, 2.8, 6.3, 7.3, and 60 mg per L, respectively. The 24-h LC_{50} for Chloramine-T determined under a variety of conditions ranged from the low of 2.8 mg per L for rainbow trout to a high of 120 mg per L for harlequin fish in soft alkaline water (pH 8.0). Acute toxicity data generated for Chloramine-T by four laboratories apparently converge. The overall body of data demonstrate that Chloramine-T is considerably less acutely toxic to fish than the presumed components of total residual chlorine (hypochlorous acid, hypochlorite ion, inorganic chloramines), a regulated body of substances, but much more toxic to fish than its stable degradate, para-toluenesulfonamide (pTSA) (Environmental Assessment... 2007). A 1983 study reported a data point for the chronic toxicity of Chloramine-T to fathead minnow (*Pimephalespromelas*) early life stage. Data for fathead minnow indicate that the 35-d NOEC is 1.1 mg per L (Machado 1983). Bills and co-workers (1988a, b) presented data indicating that time-independent LC_{50} values were statistically similar to 96-h LC_{50} values in fish. These data suggest that 96-h LC_{50} values may be useful in evaluating chronic toxicity of Chloramine-T to fish (Environmental Assessment... 2007).

In order to assess exposure to or effects of environmental pollutants on aquatic ecosystems, the following suite of fish biomarkers may be examined: biotransformation enzymes (phase I and II), oxidative stress parameters, biotransformation products, stress proteins, metallothioneins (MTs), MXR proteins, hematological parameters, immunological parameters, reproductive and endocrine parameters, genotoxic parameters, neuromuscular parameters, physiological, histological and morphological parameters (Oost et al. 2003). All fish biomarkers are evaluated for their potential use in environmental risk assessment programs, based upon six criteria. The phase I enzymes (e.g. hepatic EROD and CYP1A), biotransformation products (e.g. biliary PAH metabolites), reproductive parameters (e.g. plasma VTG) and genotoxic parameters (e.g. hepatic DNA adducts) are currently the most valuable fish biomarkers. The use of biomonitoring methods in the control strategies for chemical pollution has several advantages over chemical monitoring. Many of the biological measurements form the only way of integrating effects on a large number of individual and interactive processes in aquatic organisms. Moreover, biological and biochemical effects may link the bioavailability of the compounds of interest with their concentration at target organs and intrinsic toxicity (Oost et al. 2003). Therefore, the aim of the present study was to examine the effects of exposure to Chloramine-T on the

muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) using oxidative stress biomarkers (level of 2-thiobarbituric acid reactive substances) and biochemical enzymes activity (alanine- and aspartate aminotransferases, lactate dehydrogenase) to observe its toxic effects. The endpoints obtained from this study will be useful to monitor the effects of disinfectant bathing with Chloramine-T for this species of fish.

MATERIALS AND METHODS

Fish. Twenty two clinically healthy rainbow trout were used in the experiments. 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±2°C and the pH was 7.5. The dissolved oxygen level was about 12 ppm with additional oxygen supply. 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, rainbow trout (n = 11) were exposed to Chloramine-T in final concentration of 9 mg per L. Control group of trout (n = 11) was handled with water from basins in the same way as Chloramine-T exposed group. Fish were bathed for 20 min and repeated three times every 3 days. Two days after the last bathing fish were sampled for study. Fish were not anesthetized before tissue sampling.

Muscle tissue isolation. Muscle tissue was excised, weighted and washed in ice-cold buffer. The minced tissue was rinsed clear of blood with cold isolation buffer and homogenized in a homogenizerH500 with a motor-driven pestle on ice. The isolation buffer contained 100 mMtris-HCl; pH of 7.2 was adjusted with HCl.

Analytical methods. 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. The specific assay conditions are presented subsequently. 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.

TBARS assay for lipid peroxidation. The level of lipid peroxidation was determined by quantifying the concentration of TBARS with the Kamyshnikov method (2004) for determining the malondialdehyde (MDA) concentration. This method is based on the reaction of the degradation of lipid peroxidation product, MDA, with TBA under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. Briefly, 0.1 mL of sample was added to 2 mL of distilled water, 1 mL of 20% TCA and 1 mL of 0.8% TBA. The mixture was heated in a boiling water bath for 10 minutes. After cooling, the mixture was centrifuged at 3,000 g for 10

minutes. The nmol of MDA per 1 mg of tissue protein was calculated by using $1.56 \cdot 10^5$ mM^{-1} cm^{-1} as extinction coefficient.

Biochemical enzymes' activity. Alanine aminotransferase (ALT, E.C. 2.6.1.2) and Aspartate aminotransferase (AST, E.C. 2.6.1.1) activities were analyzed spectrophotometrically by standard enzymatic method (Reitman and Frankel 1957). The colorimetric method of Sevela and Tovarek (1959) was used for the determination of lactate dehydrogenase (LDH, E.C. 1.1.1.27) activity.

Statistical analysis. The mean ±S.E.M. values was calculated for each group to determine the significance of inter group difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). Significance of differences between the oxidative stress biomarkers level (significance level, $p < 0.05$) was examined using the Mann–Whitney *U* test. Correlations between parameters at the set significance level were evaluated using Spearman's correlation analysis (Zar 1999). All statistical calculation was performed on separate data from each individual with STATISTICA 8.0.

RESULTS

Influence of Chloramine-T on lipid peroxidation biomarker, measured as 2-thiobarbituric acid reactive substances in the muscle tissue of rainbow trout are presented in Fig. 1A. Non-significantly lower TBARS level (by 6%, $p > 0.05$) in fish disinfected by Chloramine-T (527.63 ± 62.73 nmol·mg^{-1} protein) compared to control group (540.98 ± 64.58 nmol·mg^{-1} protein) was observed (Fig. 1A).
A non-significant decrease of ALT (from 15.08 ± 2.05 to 14.18 ± 1.81 µmol pyruvate·h^{-1}·mg^{-1} protein) and AST activity (from 25.32 ± 2.95 to 22.82 ± 1.52 µmol pyruvate·h^{-1}·mg^{-1} protein) in the muscle tissue of the trout disinfected by Chloramine-T was found (Fig. 1B). LDH activity in the muscle tissue of chloramine-T-exposed trout were significantly decreased by 339% ($p = 0.017$) compared to controls (from 42.52 ± 4.07 to 28.61 ± 2.71 µmol pyruvate·h^{-1}·mg^{-1} protein) (Fig. 1B).
Several correlations between checked parameters were found (Fig. 2). Muscle TBARS level is correlated positively with ALT ($r = 0.858$, $p = 0.001$) and AST ($r = 0.896$, $p = 0.000$) in unhandled control group (Fig. 2A). LDH activity is correlated positively with ALT ($r = 0.689$, $p = 0.019$) and AST ($r = 0.852$, $p = 0.000$) in the muscle tissue of rainbow trout exposed by Chloramine-T (Fig. 2B).

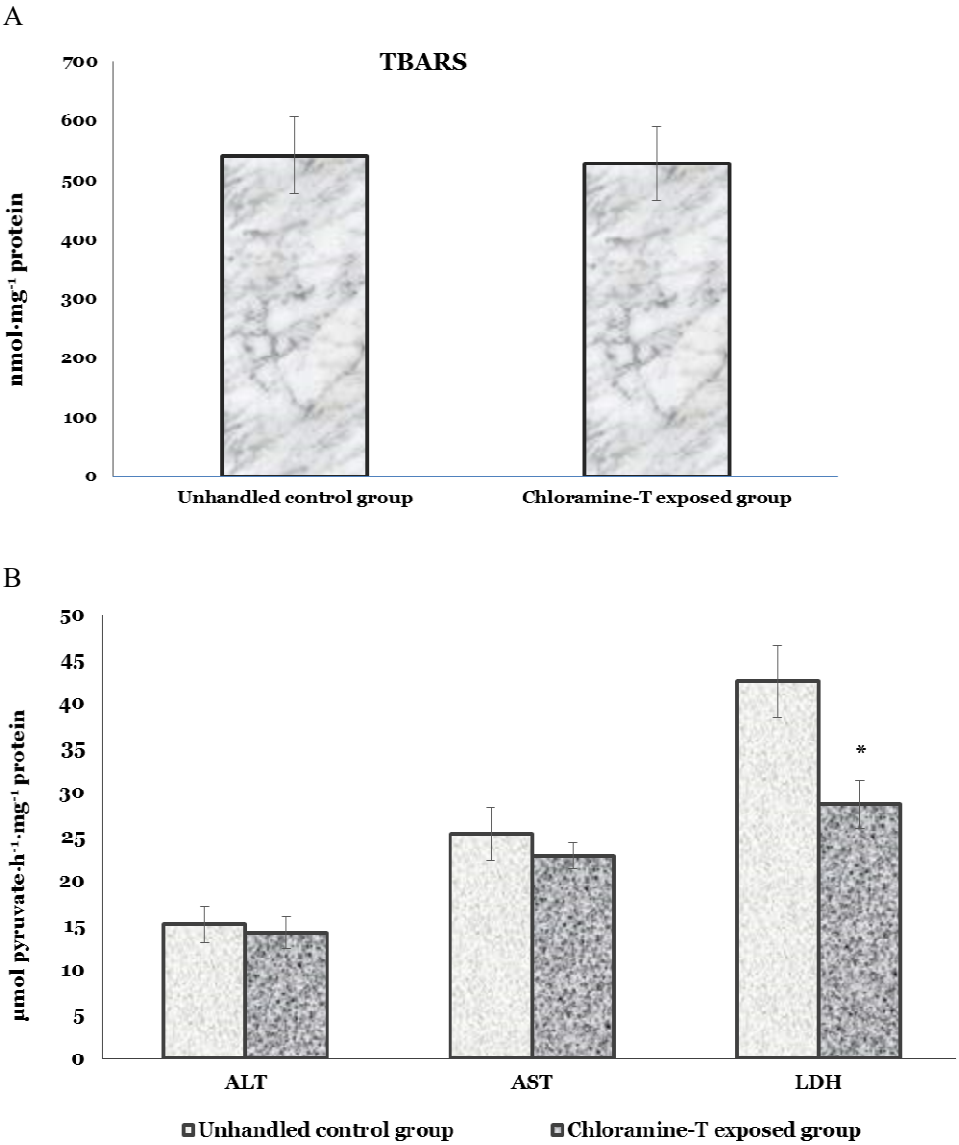
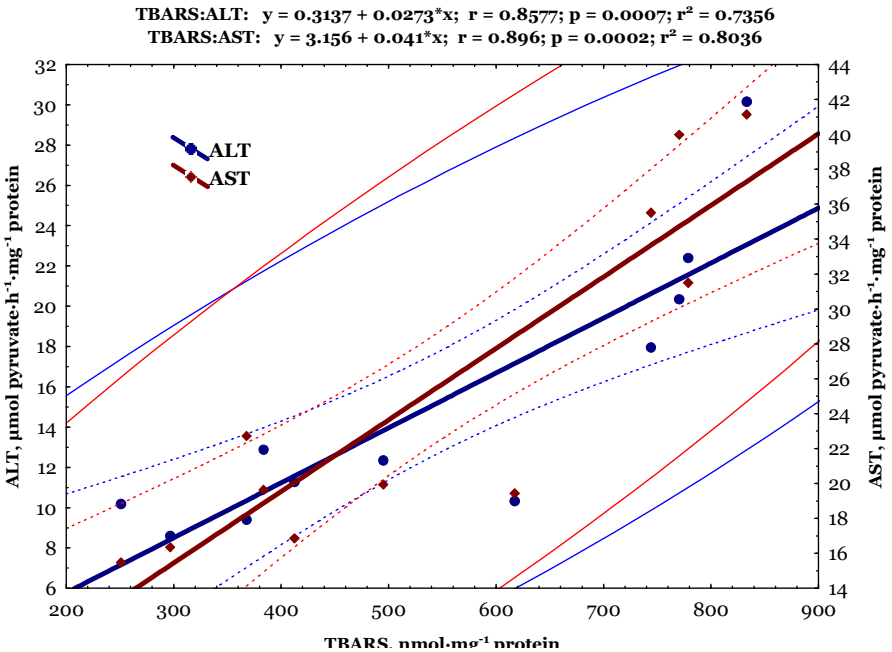


Fig. 1. The lipid peroxidation biomarker, measured as 2-thiobarbituric acid reactive substances (TBARS, A), as well as alanine and aspartate aminotransferases, lactate dehydrogenase activity (B) in the muscle tissue rainbow trout (*Oncorhynchus mykiss* Walbaum) disinfected by Chloramine-T

* the significant difference was shown as $p < 0.05$ when compared control and Chloramine-T exposed groups

A



B

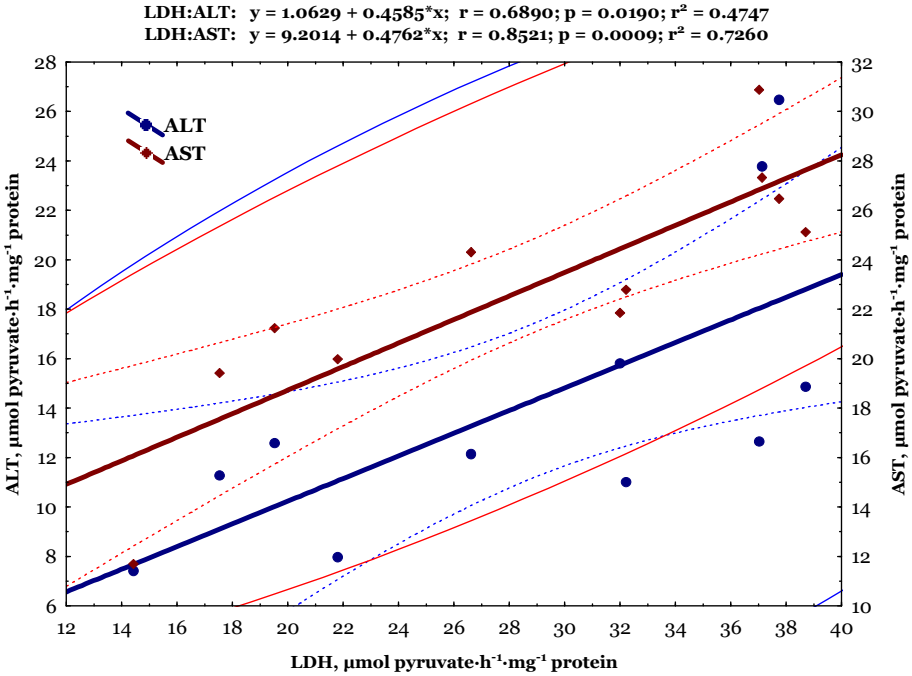


Fig. 2. Correlations between TBARS, ALT and AST activity in the muscle tissue of unhandled control group (A), and LDH, ALT and AST activity in the muscle tissue of trout disinfected by chloramine-T

DISCUSSION

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are found in the liver, heart, skeletal muscle, kidney, pancreas, spleen, erythrocyte, brain and gills (Banaee et al. 2011). When diseases or injuries affect these tissues, the cells are destroyed and these enzymes are released into plasma. Xenobiotics in fish liver can be metabolized to metabolites by cytochrome P450 monoxygenase and during this process, reactive oxygen species (ROS) are generated. The increase in intracellular levels of ROS may lead to lipid peroxidation resulting in an increased permeability of liver cell membrane. As a result, liver enzymes including AST and ALT are released into plasma. Lactate dehydrogenase (LDH) is an enzyme found in almost all body tissues, such as heart, kidneys, liver, skeletal muscle, brain, erythrocyte and gills (Banaee et al. 2011). LDH measurement is used to detect tissue disorders and as an aid in the diagnosis of tissue damage (Banaee et al. 2011).

Our results showed that Chloramine-T bathing caused the decrease of the lipid peroxidation with non-significant decrease of ALT and AST activity as well as significantly decreased LDH activity (Figs 1A and 1B). Moreover, lipid peroxidation level is linked with ALT ($r = 0.858$, $p = 0.001$) and AST activity ($r = 0.896$, $p = 0.000$) in the muscle tissue of unhandled control group (Fig. 2A). In the muscle tissue of trout disinfected by chloramine-T, LDH activity is correlated positively with ALT ($r = 0.689$, $p = 0.019$) and AST ($r = 0.852$, $p = 0.000$) (Fig. 2B).

Skeletal muscle of fishes plays an important role in the post-exercise processing of lactate through the gluconeogenic and glycogenic pathways, a situation very different from that in mammals, where the enzymatic machinery for regenerating glucose is primarily found in the liver (Suarez et al. 1986, Moon 1988, Gleeson 1996). The muscle of fish is thus more multi-functional than that of mammals, possessing a wider array of enzymes and greater inherent metabolic flexibility than mammalian muscle, including a greater potential for biosynthesis (Gleeson 1996). In fact, fish skeletal muscle has been shown to sequester lactate post-exercise (Gleeson 1996), facilitating the regeneration of glucose within the muscle and enhancing post-exercise recovery (Torres et al. 2012).

Skeletal muscle acts as a clearing house for lactate produced in brain, heart and liver during anaerobiosis. Lactate is taken up by muscle and converted to pyruvate by LDH. Pyruvate reaching the muscle *via* the bloodstream and that produced during glycolytic activity in the muscle is processed by the pyruvate dehydrogenase (PDH) pathway within the mitochondrion to produce acetaldehyde and CO_2; acetaldehyde is converted to ethanol within the cytosol, and ethanol can then diffuse out of the cell to be excreted at the gills (Shoubridge and Hochachka 1980). Indeed, LDH is an enzyme participated in anaerobic pathway of carbohydrate metabolism. The increase of LDH activity is a diagnostic index widely used to recognize increases of anaerobic metabolism resulting from depletion of energy under anaerobic and environmental stress conditions. The increase of LDH activity can be attributed to the conversion of accumulated pyruvate into lactate which is transported through muscle to hepatopancreas and regenerated glucose and glycogen to supply energy fish exposed to xenobiotics. In other words, the increase of LDH activity in liver and muscle reflects a possible improvement in tissue glycolytic capacity (Banaee 2013).

In our study, correlation between pyruvate recovery through aminotransferases (ALT and AST activity) and lactate conversion to pyruvate through LDH was shown (Fig. 2B). In our previous study (Tkachenko et al. 2012), Chloramine-T bathing markedly decrease aldehydic and ketonic derivatives of oxidative protein, and aminotransferases activity only in rainbow trout liver, 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 aldehydic and ketonic derivatives of oxidative protein could modify lactate and pyruvate levels, aminotransferases and lactate dehydrogenase activities, principally causing increased enzymes activity due to oxidative stress in the liver of chloramine-exposed fish (Tkachenko et al. 2012).

In our study, we used Chloramine-T as disinfectant agent in concentration of 9 mg per L. For example, Boran and Altinok (2014) assessed the effects of therapeutic, and higher concentrations of Chloramine-T on the antioxidant enzyme system and genetic structure in juvenile rainbow trout *Oncorhynchusmykiss* (Walbaum). Red blood cells acetylcholinesterase, Δ-aminolevulinic acid dehydratase, paraoxonase and liver glutathione S-transferase activity were increased at 10 and 20 mg per L Chloramine-T-exposed fish, while they were decreased at 30 mg per L Chloramine-T-exposed fish. On the other hand, liver catalase activity and liver protein levels increased at 10 mg per L and decreased at 20 and 30 mg per L concentrations of Chloramine-T. Liver superoxide dismutase activity decreased at 10 mg per L and 20 mg per L Chloramine-T and increased at 30 mg per L of Chloramine-T. Compared to control, comet assay indicated that Chloramine-T did not cause significant DNA damage to red blood cells of the fish. Results indicate that 10 or 20 mg per L Chloramine-T can be safely used to prevent or treat external parasitic and bacterial infection of rainbow trout (Boran and Altinok 2014).

Nonetheless, in study of Leef and co-workers (2007), treatment with Chloramine-T at 10 mg per L appeared to briefly mitigate the rise in standard metabolic rates (RS), as there was an approximately 30% drop (not statistically significant) in RS following treatment. In rainbow trout *Oncorhynchus mykiss*, acute Chloramine-T exposure at 9 mg per L in fresh water induces both respiratory and acid-base disturbances that may be directly related to hyperventilation (Powell and Perry 1997) and an increase in bronchial mucus production due to the irritant effect on the gills (Powell and Perry 1996). Oxygen consumption rates also increases following Chloramine-T exposure at 9 mg per L (Powell and Perry 1999).

Accumulating evidence has shown that Chloramine-T causes oxidative stress by inducing the generation of reactive oxygen species (ROS) (Tatsumi and Fliss 1994, Sakuma et al. 2009, Stanley et al. 2010). The data suggest that HOCl and monochloramine can increase endothelial permeability by causing very rapid cytoskeletal shortening and cell retraction, possibly as a result of the oxidation of intracellular sulfhydryls (Tatsumi and Fliss 1994). Sakuma and co-workers (2009) assessed the influence of monochloramine on the conversion of xanthine dehydrogenase into xanthine oxidase in rat liver *in vitro*. 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 xan-

thine 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 (Sakuma et al. 2009).

Moreover, HOCl and related oxidants such as N-chloramines may damage DNA (Stanley et al. 2010). There is a strong link between chronic inflammation and the incidence of many cancers caused by HOCl and related oxidants such as N-chloramines (Stanley et al. 2010). Stanley and co-workers (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 Nα-acetyl-histidine (His-C), Nα-acetyl-lysine (Lys-C), glycine (Gly-C), taurine (Tau-C), and ammonia (Mono-C). Treatment of DNA and related materials with HOCl and Nα-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 (Stanley et al. 2010).

Chloramine-T was used to mimic the effects of free radicals and active oxygen compounds implicated in brain ischemia and head trauma. Previous studies have shown that free radicals produce both synaptic and postsynaptic damage in guinea pig hippocampal brain slices. Chloramine-T (25 to 500 μM (7.0 to 140.8 μg/mL)) decreased both the population spike and population postsynaptic potential. However, the ability of the population postsynaptic potential was not impaired by the treatment. These studies suggest that oxidation reactions account for the synaptic component of free radical-induced damage in the nervous system but not the postsynaptic effects (Pellmar and Neel 1989).

There are many evidences that Chloramine-T could be toxic for fish. For example, effect of prophylactic Chloramine-T treatment on growth performance and condition indices of rainbow trout (*Oncorhynchusmykiss*) have been studied by Sanchez and co-workers (1997). Using a 24-tank replicate growth assay system, rainbow trout (average weight 98 g) were exposed twice weekly to Chloramine-T at 10 mg per L for 1 hour, throughout an 11-week growth trial and compared to matched controls. Fish were fed ad libitum without feed wastage to assess appetite and feed conversion. Growth parameters were assessed every 3 weeks, at the end of weeks 3, 6, 9, and 11. Chloramine-T treatment was not associated with either clinical disease or mortality. Final weight and specific growth rate were significantly impaired during the growth trial in the groups of fish treated with Chloramine-T compared to controls. This was attributed to a significant depression of feed conversion efficiency and to a minor depression in appetite in treated fish (Sanchez et al. 1997). Chloramine-T treatment (at 10 mg per L for 1 h twice weekly for 11 weeks) was not associated with either clinical disease or mortality. However, by the end of the trial, growth (based on body weight) of treated fish was significantly suppressed com-

pared with control fish. Growth suppression was attributed to a significant reduction of feed conversion efficiency in treated fish. Based on specific growth rates, Chloramine-T had an early negative effect on growth. The effect was diminished in later weeks (although not completely lost), which suggests some degree of compensation by the fish to the chemical agent (Sanchez et al. 1996).

Sanchez and co-workers (1998) have concluded that although Chloramine-T and formalin may continue to be useful in the aquaculture industry they cause potentially harmful alterations to fish skin. Repeated treatment, once weekly for 4 weeks, caused to increase numbers of highly dense vesicles within the apical portions of epithelial cells. The epidermal mucous cells of Chloramine-T-treated fish were significantly smaller than in controls. This effect was not noted in formalin-treated fish. Treatment with either chemical resulted in a significantly thinned epidermis (Sanchez et al. 1998). The use of Chloramine-T (10 mg per L, treating twice weekly for 11 weeks with a one-hour static bath) evoked a slight increase in mean lamellar width, but it did not induce lamellar oedema, lamellar fusion, tissue infiltration, epithelial hyperplasia, chloride cell metaplasia or thrombosis of pillar channels in treated fish. Treatment caused a trend towards an increased number of mucous cells on lamellae, associated with a significant shift from neutral mucin to acid mucin production based on histochemical characteristics (Sanchez et al. 1997).

The stress response in healthy juvenile rainbow trout after repetitive intermittent treatment with Chloramine-T or formalin also have evaluated by Sanchez and co-workers (1997). Tanks of healthy juvenile rainbow trout were exposed to Chloramine-T (10 mg per L; 40 fish) or formalin (200 mg per L; 40 fish) for 1 h once per week for 4 weeks. The effect of this treatment on the primary stress response was evaluated by measuring circulating cortisol levels with a radioimmunoassay technique. Blood cortisol levels were analyzed at 1, 24, and 96 h after each treatment and compared with pre-exposure baseline values and with values obtained from sham-treated fish. At 1 h, fish in all treatment categories had elevated cortisol levels compared with baseline values, but values in those fish treated with chemicals were no different from those that were sham treated. Cortisol levels returned to near baseline by 24 h after treatment. In a second experiment, the effect of twice weekly 1-h exposure to Chloramine-T (10 mg per L) on secondary stress indices of rainbow trout was probed during an 11-week growth trial by measuring haematocrit, plasma glucose, sodium, and chloride levels in treated, untreated, and sham-treated fish. No evidence of a secondary stress response could be detected in fish treated with Chloramine-T when they were compared with either control group. It is concluded that intermittent exposure to Chloramine-T at 10 mg per L does not elicit a primary or secondary stress response in rainbow trout and that stress is not the mechanism responsible for growth deceleration in treated fish (Sanchez et al. 1997).

The effects of repeated intermittent exposure of healthy rainbow trout fingerlings to sublethal concentrations of Chloramine-T (0, 5, 10, or 20 mg per L) twice weekly in 1-h pulses at 11°C for 4 weeks in a replicate-tank facility were examined by Powell and co-workers (1995). Gills were excised from subsamples of fish before exposure and at the end of the 4-week experimental period. The gill epithelium from fish treated with 10 and 20 mg per L Chloramine-T appeared swollen and vacuolated, with extensive intercellular oedema. There was a significant reduction in the number

of lamellar mucous cells and an apparent increase in the numbers of chloride cells. Chloride cells from both the base of the lamella and the lamellar surface of gills exposed to Chloramine-T had an increase in the area of the apical plasmalemma after treatment with 10 and 20 mg per L, and a reduction in the thickness of the apical plasmalemma-associated glycocalyx. These morphological changes are consistent with a compensatory mechanism for the remedial uptake of ions, suggesting that Chloramine-T increased epithelial ion permeability coincident with a possible influx of water leading to intercellular oedema. Chloride cell proliferation and intercellular oedema may also have affected gas exchange across the branchial epithelium (Powell et al. 1995).

Powell and co-workers (1998) also have examined the physiological effects of repeated exposure to 9 mg per L Chloramine-T, a common aquaculture disinfectant for rainbow trout using a graded hypoxic challenge. Using an extracorporeal circulation, continuous measurements of blood PO_2, PCO_2 and pH were made and correlated with decreasing water PO_2. Ventilation amplitude and frequency were also monitored. Following the graded hypoxic challenge, the gills were removed and processed for microscopy for morphometric measurements and the determination of the number of bronchial mucous cells. Fish treated with Chloramine-T exhibited a higher arterial PO_2 during hypoxia between 10.1 and 11.2 kPa when compared with untreated controls; there were no differences in arterial PCO_2 or pH between the two groups. Chloramine-T-treated fish had an elevated pre-hypoxic ventilation frequency as compared with the controls. However, under the graded hypoxia, control fish elevated their ventilation frequency, whereas Chloramine-T-treated fish did not. Both Chloramine-T-treated and control fish increased their ventilation amplitude during the graded hypoxia and there were no differences between control and Chloramine-T-treated fish. The fish treated with Chloramine-T had a reduced thickness of the gill epithelial blood-to-water diffusion barrier but higher numbers of mucous cells as compared with controls. Powell and co-workers (1998) 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 (Powell et al. 1998).

Sirri and co-workers (2013) have tested two concentrations of water disinfectants, chloramine T and peracetic acid, on *Garra rufa* to ascertain possible exposure damage to the epidermis and gills. Fish were exposed to 2 mg per L and 10 mg per L of chloramine T and to 15 µL per L and 45 µL per L of peracetic acid in a 40-minute static bath up to six times a day for one week. The epidermis and gills were checked for histological changes and the number of epidermal mucous cells, club cells and taste buds were quantified; mucous cells were also characterized histochemically to detect alterations in mucin production. No mortality or severe histological changes were found in treated or control fish. Cell count showed a significant increase ($p < 0.05$) in mucous cells (mean 49.1 ± 6.7 vs 37.0 ± 13.1 of controls) in animals treated with peracetic acid independently of the dose. Club cell number showed a significant ($p<0.05$) decrease in fish treated with 2 mg per L of chloramine T (mean 74.3 ± 15.6) and with 45 µL per L of peracetic acid (mean 78.17 ± 10.5) compared to controls (mean 107.0 ± 19.2). Histochemical evaluation of mucous cells did not reveal changes in mucin type in fish exposed to the two disinfectants. The results suggest

a good tolerability of *Garra rufa* to the two disinfectants at the concentrations tested (Sirri et al. 2013).
In our study, no mortality or severe biochemical changes were found in treated or control fish.Our results showed that Chloramine-T bathing caused the decrease of the lipid peroxidation with non-significant decrease of ALT and AST activity as well as significantly decreased LDH activity.

CONCLUSIONS

Chloramine-T in dose of 9 mg per L has no influence on the level of lipid peroxidation in the muscle tissue of rainbow trout. Chloramine-T markedly affects on lactate and pyruvate metabolism and resulted to decrease of LDH activity. The decrease of LDH activity can be attributed to the decrease of conversion of accumulated pyruvate into lactate which is transported through muscle to hepatopancreas and regenerated glucose and glycogen to supply energy fish exposed to xenobiotics. In other words, the decrease of LDH activity in liver and muscle reflects a possible decrease in tissue glycolytic capacity (Banaee 2013). These parameters could be effectively used as potential biomarkers of Chloramine-T toxicity to the fish in the warning signal for pharmaceutical exposure to aquatic organisms. Our studies indicated that Chloramine-T in dose of 9 mg per L could at least partly attenuate oxidative stress and can be used for prophylactic disinfecting treatment of rainbow trout. However, more detailed studies on using of these specific biomarkers to monitor the disinfectant treatment in aquaculture are needed.

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ZMIANY BIOCHEMICZNE W TKANCE MIĘŚNIOWEJ PSTRĄGA TĘCZOWEGO (*ONCORHYNCHUS MYKISS* WALBAUM) PO KĄPIELACH DEZYNFEKUJĄCYCH Z CHLORAMINĄ-T

Streszczenie

Chloramina-T jest szeroko stosowanym środkiem dezynfekcyjnym i terapeutycznym do leczenia chorób skrzeli ryb w wodach słodkich i morskich. Jednak pomimo szerokiego stosowania tego środka, tylko w niewielu badaniach analizowano jego toksyczność dla ryb (Powell i Harris 2004). W związku z tym celem pracy było zbadanie wpływu dezynfektanta chloraminy-T na tkankę mięśniową pstrąga tęczowego (*Oncorhynchus mykiss* Walbaum) z wykorzystaniem biomarkerów stresu oksydacyjnego (poziom produktów reagujących z kwasem 2-tiobarbiturowym, aldehydowe i ketonowe pochodne oksydacyjnej modyfikacji białek) oraz przemian metabolicznych (aktywność aminotransferaz alaninowej i asparaginianowej, dehydrogenazy mleczanowej, stężenie mleczanu i pirogronianu). Uzyskane wyniki końcowe będą przydatne do monitorowania skutków dezynfekujących kąpieli z chloraminą-T dla tego gatunku ryb. Nasze wyniki wskazują, że chloramina-T znacznie obniża peroksydację lipidów na tle zmniejszenia aktywności aminotransferaz alaninowej i asparaginianowej oraz dehydrogenazy mleczanowej. Ponadto obniżona aktywność dehydrogenazy mleczanowej spowodowała zmniejszenie aktywności aminotransferaz. Zatem mięśnie szkieletowe ryb odgrywają ważną rolę w obróbce mleczanu przez glukoneogenezę i glikogenezę. Chloramina-T w dawce 9 mg na litr może przynajmniej częściowo złagodzić stres oksydacyjny w tkance mięśniowej pstrąga tęczowego i może być stosowana do dezynfekcji tego gatunku ryb. Konieczne są jednak bardziej szczegółowe badania dotyczące korzystania z tych specyficznych biomarkerów do monitorowania dezynfekcji w akwakulturze.