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Research Article

Inhibition Effect of Some Chemical Anesthetics and Hypothermia on the Activity of Glucose 6-phosphate Dehydrogenase From Rainbow Trout (*Oncorhynchus mykiss*) Erythrocytes In Vivo

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Abstract: Many chemical anesthetics and drugs have been used in fish stripping, surgery and transportation, but their undesirable side effects on the body metabolism during treatment are not usually taken into consideration.

In this study, we investigated the inhibition effect of some chemical anesthetics, such as Tranquil and tricaine methanesulfonate (MS-222), and hypothermia on glucose 6-phosphate dehydrogenase (G6PDH) activity. The control, initial, 1 h and 4 h values were 41.334 ± 3.190 EU (g Hb)$^{-1}$, 34.586 ± 5.086 EU (g Hb)$^{-1}$, 40.768 ± 3.430 EU (g Hb)$^{-1}$ and 41.957 ± 3.157 EU (g Hb)$^{-1}$, respectively, in hypothermia. These values were 39.470 ± 2.250 EU (g Hb)$^{-1}$, 35.807 ± 2.000 EU (g Hb)$^{-1}$, 32.756 ± 7.822 EU (g Hb)$^{-1}$ and 34.882 ± 3.452 EU (g Hb)$^{-1}$ with Tranquil and 52.910 ± 2.490 EU (g Hb)$^{-1}$, 27.044 ± 2.750 EU (g Hb)$^{-1}$, 39.901 ± 4.477 EU (g Hb)$^{-1}$ and 42.629 ± 2.593 EU (g Hb)$^{-1}$ with MS-222, respectively. Hypothermia, Tranquil and MS-222 showed inhibition effects on G6PDH activity. The inhibition effects of hypothermia and Tranquil were not statistically significant ($P > 0.05$), but MS-222 caused statistically quite high inhibition initially ($P < 0.01$). The inhibition effect of MS-222 diminished 1 h and 4 h after treatment ($P < 0.05$).

Key Words: *Oncorhynchus mykiss*, anesthetics, inhibition, G6PDH.

Bazı Kimyasal Anestezikler ve Soğukla Muamelenin Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Eritrositlerindeki Glikoz 6-fosfat Dehidrogenaz Enzimi Aktivitesi (In Vivo) Üzerine Inhibisyon Etkileri

Özet: Balıkların sağımında, cerrahisinde ve taşınmasında birçok kimyasal anestezik ve ilaçlar kullanılmaktadır. Fakat muamele esnasında anasteziklerin balık metabolizması üzerine olan olumsuz etkileri çoğunlukla dikkate alınmamaktadır.

Bu çalışmada, Tranquil, tricaine methanesulfonate (MS-222) gibi kimyasal anestezilerin ve soğukla muamelenin glikoz 6-fosfat dehidrogenaz (G6PDH) aktivitesi üzerine inhibisyon etkileri araştırıldı. Kontrol, başlangıç, 1. saat ve 4. saatte enzim aktiviteleri sırasıyla soğukla muamelede 41,334 ± 3,190 EU (g Hb)$^{-1}$, 34,586 ± 5,086 EU (g Hb)$^{-1}$, 40,768 ± 3,430 EU (g Hb)$^{-1}$ ve 41,957 ± 3,157 EU (g Hb)$^{-1}$, Tranquilde 39,470 ± 2,250 EU (g Hb)$^{-1}$, 35,807 ± 2,000 EU (g Hb)$^{-1}$, 32,756 ± 7,822 EU (g Hb)$^{-1}$ ve 34,882 ± 3,452 EU (g Hb)$^{-1}$, MS-222'de ise 52,910 ± 2,490 EU (g Hb)$^{-1}$, 27,044 ± 2,750 EU (g Hb)$^{-1}$, 39,901 ± 4,477 EU (g Hb)$^{-1}$ ve 42,629 ± 2,593 EU (g Hb)$^{-1}$ olarak tespit edildi.

Hypotermia, Tranquil ve MS-222, G6PDH aktivitesi üzerinde inhibisyon etkisi gösterdi. Hypotermia ve Tranquilin inhibisyon etkileri istatistiki olarak önemsiz ($P > 0,05$), fakat MS-222'nin inhibisyon etkisi başlangıçta istatistiki olarak oldukça yüksek bulundu ($P < 0,01$). MS-222'nin inhibisyon etkisi muameleden sonraki 1. ve 4. saatte azaldı ($P < 0,05$).

Anahtar Sözcükler: *Oncorhynchus mykiss*, anastezik, inhibisyon, G6PDH.

Introduction

Glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate: $NADP^+$ oxidoreductase EC 1.1.1.49: G6PDH) is the first enzyme in the pentose phosphate pathway. The main physiological function of G6PDH is the production of NADPH and ribose 5-phosphate, which are essential for reductive biosynthesis and nucleic acid synthesis (1). The major role of NADPH in erythrocytes is the regeneration of reduced glutathione, which prevents hemoglobin denaturation, preserves the integrity of red

blood cells' membrane sulfhydryl groups, and detoxifies hydrogen peroxide and oxygen radicals in and on the red blood cells (2,3).

At the cellular level a continuous supply of reducing equivalents in the form of NADPH is essential for growth and proliferation processes, serving as they do as hydrogen and electron sources for a variety of reductive biosynthetic reactions, including the synthesis of fatty acids and cholesterol (4,5), both of which are necessary for membranogenesis.

It is generally recognized that the cell has 4 major NADPH-production systems, corresponding to the activities of 4 cytoplasmatic enzymes: G6PDH, 6-phosphogluconate dehydrogenase (6PGDH) belonging to the pentose phosphate pathway, malic enzyme (ME) and NADP-dependent isocitrate dehydrogenase (NADP-IDH). The G6PDH reaction is important in metabolic control (5-7).

Several authors have established that in mammals the activity of G6PDH alters according to the metabolic, hormonal and nutritional state of the animal (6,8), whilst others have reported similar responses in fish in varying nutritional conditions (9,10). On the other hand, the inhibitory effects of some antibiotics on G6PDH from human erythrocytes have been investigated (11).

We have been unable to find any published information on the influence of chemical anesthetics or hypothermia on the activity of G6PDH in fish. Therefore we investigated the inhibition effects of Tranquil, tricaine methanesulfonate (MS-222) and hypothermia on G6PDH activity.

Materials and Methods

Chemicals and hypothermia

Chemicals of analytical grade from Sigma and Merck were used. The treatment concentrations of Tranquil and MS-222 were 12 mg/l and 100 mg/l, respectively (12). Cold fresh water (0-1 °C) was used for hypothermia.

Experimental design and blood sampling

Forty fish were randomly selected (100 ± 20 g weight) for each group. Prior to the experiment, the fish in each group were kept in 1 x 1.2 m (wide-deep) fiber-glass tanks for 1 month. The fish were fed commercial trout food. The tanks were supplied with fresh water at a flow rate of 0.01-1 (l/min)/kg body weight. At the end of the adaptation period, the fish were put into 3 tanks each containing a different kind of anesthetic initially. Then the other fish were transferred to 3 fiber-glass tanks. After 1 h and 4 h blood samples were taken from 10 fish randomly chosen from each group. The blood specimens were sampled from the caudal vein using a 10 ml plastic-heparinized syringe, put into tubes and centrifuged at 2500 x g for 15 min. The plasma and leukocyte coat were removed. After the red cells were washed with KCl solution (0.16 M) 3 times, the samples were centrifuged at 2500 x g each time and the supernatants were removed. The erythrocytes were hemolyzed with 5 vol. of ice-cold water and centrifuged at +4 °C, 10000 x g, for 30 min to remove the ghosts and intact cells (13,14).

Measurements of G6PDH activity

G6PDH activity was measured according to Beutler's method; 100 µl of 1 M Tris-HCl, 5 mM EDTA solution at pH 8 + 100 µl of 0.1 M $MgCl_2$ + 100 µl of 2 mM $NADP^+$ + 20 µl of 1:20 hemolysate + 580 µl of H_2O incubated at 37 °C for 10 min and 100 µl of 6 mM glucose 6-phosphate (G6P) was added and then absorbance was measured against distilled water at 340 nm in a spectrophotometer (Shimadzu UVmini – 1240). This method depends on the reduction of 2 mM $NADP^+$ by G6PDH in the presence of G6P. The activity was measured by monitoring the increase in absorption at 340 nm due to reduction of $NADP^+$. One enzyme unit was regarded as the reduction of 1 µM of $NADP^+$ per minute. Specific enzyme activity is expressed in terms of EU (g $Hb)^{-1}$. Hemoglobin was measured according to the cyanmethemoglobin method (15).

The data obtained were analyzed made by t-test and given as $\bar{X} \pm SE$.

Results

The inhibition effects of Tranquil, MS-222 and hypothermia on G6PDH activity are summarized in Table.

When the G6PDH activity of the control groups are compared with those of the hypothermia and Tranquil treatment groups, there were low inhibitions and they were not statistically significant ($P > 0.05$). However, MS-222 showed statistically quite high inhibition initially ($P < 0.01$). The inhibition effect of MS-222 diminished 1 and 4 h after treatment ($P < 0.05$).

Table. The effects of Tranquil, MS-222 and hypothermia on G6PDH activity in rainbow trout (*Oncorhynchus mykiss*) erythrocytes.

Anesthetics	Time	N	$\bar{X} \pm SE$ [EU (g Hb)$^{-1}$]
	Control	10	41.334 ± 3.190
Hypothermia	Initial	10	34.586 ± 5.086
	1 h	10	40.768 ± 3.430
	4 h	10	41.957 ± 3.157
	Control	10	39.470 ± 2.250
Tranquil	Initial	10	35.807 ± 2.000
	1 h	10	32.756 ± 7.822
	4 h	10	34.882 ± 3.452
	Control	10	52.910 ± 2.490
MS-222	Initial	10	27.044 ± 2.750**
	1 h	10	39.901 ± 4.477*
	4 h	10	42.629 ± 2.593*

Mean values with different letters are significantly different at *P < 0.05, **P < 0.01

As shown in Figure, G6PDH activity in the hypothermia group was low initially but increased after 1 and 4 h. In contrast, MS-222 and Tranquil diminished G6PDH activity initially and after 1 and 4 h.

Discussion

Three different anesthetics were used to anesthetize rainbow trout, and their effects on the G6PDH activity of this species were investigated.

Winzer et al. (16) investigated the role of G6PDH in oxidative stress responses in isolated intact living hepatocytes of immature female and male European flounder (*Platichthys flesus* L.). Hepatocytes were exposed to sublethal concentrations of effective prooxidants such as hydrogen peroxide (H_2O_2), benzo[a]pyrene (B[a]p) and nitrofurantoin (NF) 17-β-estradiol during culture. It was shown that there was significant inhibition of G6PDH activity by all oxidative stressors and 17-β-estradiol in both sexes of fish independent of the culture conditions, but inhibition was stronger in the cells of females than in the cells of males.

Çiltaş et al. (17) emphasized that $CuSO_4$ and chloramine-T inhibited G6PDH from rainbow trout erythrocytes. On the other hand, in some studies on human G6PDH, it was shown that some drugs used in human medicine inhibited G6PDH activity (18,19). Additionally, in a study on G6PDH in human erythrocytes, it was reported that N,O-dimethyl hydroxylamine led primarily to inhibition of G6PDH activity (20).

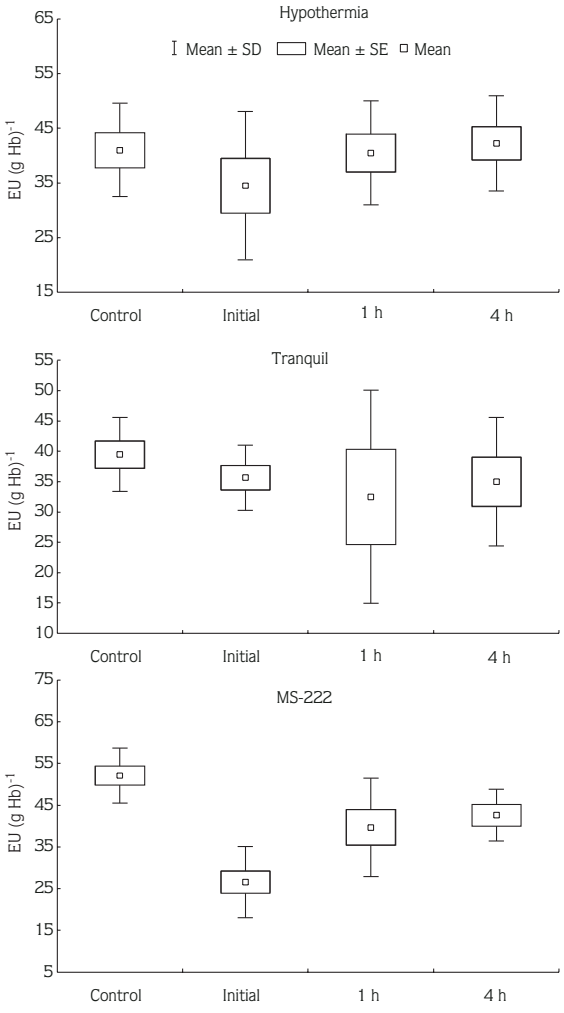

Figure. Effects of anesthetics on G6PDH activities in erythrocytes of rainbow trout.

G6PDH is not only inhibited by some drugs, and toxic and chemical substances but also some anesthetics. Likewise, Gabryelak et al. (21) reported that at 16 °C MS-222 in a concentration of 70 mg/l caused an enhancement in the superoxide dismutase (SOD) and peroxidase activities and a decrease in the catalase activity in *Cyprinus carpio* and *Dicentrarchus labrax*. The results show that MS-222 has an adverse effect on peroxidase activities. In an investigation on brook trout (*Salvelinus fontinalis*) exposed to a 30.0 mg/l solution of quinaldine sulfate or a 112.5 mg/l solution of tricaine (MS-222) for 5 min the in vitro hydroxylation of benzo(a)pyrene decreased (22). These investigations show that MS-222 may have an adverse effect on some enzymes activities. Similarly, we found that MS-222 also inhibited G6PDH enzyme activity in rainbow trout erythrocytes. This inhibition was quite high initially ($P < 0.01$) but was high after 1 and 4 h ($P < 0.05$).

Kurtuluş and Tuncer (23) investigated the effect of different doses of halothane on the G6PDH activity of mouse liver. They found that increasing the halothane anesthesia dosage in the mouse induced liver G6PDH activity. Kumar et al. (24) reported that trichloroethylene (TCE), an anesthetics agent, caused a significant decrease ($P < 0.05$) in total epididymal sperm count, sperm motility, specific activities of G6PDH and 17 β hydroxy steroid dehydrogenase (17 β HSD) with a concomitant decrease in serum testosterone concentrations and reduced male reproductive efficiency in TCE-inhaled rats. These investigations show that some anesthetics may inhibit G6PDH activity. The results of our investigation are consistent with those of the other researchers mentioned.

There are many studies on the inhibition of G6PDH activity but few on the effects of anesthetics on this enzyme in fish. Many anesthetics have been used in fish culture but some cause adverse effect on fish. Therefore, anesthetics should be used with care.

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