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RESEARCH PAPER

Effects of Surgical Implanted Dummy Ultrasonic Transmitters on Biochemical Parameters of Silver Carp Hypophthalmichthys Molitrix

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Abstract

To gain a better understanding of the ultrasonic telemetry research and apply the ultrasonic telemetry into freshwater fish, we estimated the influence of surgical implantation of ultrasonic transmitters on the biochemical parameters of silver carp. The experiment was repeated thrice. SPSS 17.0 software was used for data processing. The results showed that The level of plasma glucose, total protein, globulin and aspartate aminotransferase (AST) in the sham and surgery groups increased significantly ($P<0.01$) 1 day after surgery, on the contrary, The level of plasma Albumin and chlorine declined significantly when compared with the control group. After 7 days, the level of total protein, globulin and AST had declined but still remained higher for sham and surgery groups than control group, whereas the level of plasma glucose had declined to normal level. The level of plasma albumin in the sham and surgery groups continued to decline and they were significantly lower than control group ($P<0.01$). After 14 days, there was no significant difference between the three groups. The level of triglycerides, cholesterol and alanine aminotransferase (ALT) showed no significant difference between the three groups in 1 day, 7 days and 14 days after surgery.

Keywords: Surgical implantation, ultrasonic telemetry, biochemical parameters, silver carp Hypophthalmichthys molitrix.

Introduction

The ultrasonic telemetry has been used in a large number of field and laboratory studies (Thorstad *et al*., 2013; Baras, 1991). However, the transmitter attachments may influence the behavior and performance of the fish (Lewis and Muntz, 1984; Mellas and Haynes, 1985). Many of the relevant researches have been done to investigate the influence and they show mixed results, some reporting changed in blood biochemical parameters and reduced swimming performance in tagged fish (Adams *et al*., 1998) and others reporting no effects (Thorstad *et al*., 2000). Discrepancies between studies are at least partially due to species and size differences. For some fish species, such as Atlantic salmon, the negative effects of transmitters have been studied in juveniles, smolts and adults. The results showed that intraperitoneal implantations had no significant effect on growth, feeding or swimming behaviour in either parr or smolts (Moore *et al*., 1990; Peake *et al*., 1997), and swimming performance and blood physiology of adult Atlantic salmon were not affected when equipped with external or body-implanted telemetry transmitters (Thorstad *et al*., 2000). But the size of dummy ultrasonic transmitters would affect the swimming performance of the Atlantic salmon (McCleave and Stred, 1975). However, these potential negative effects are still unknown for a number of species, especially in freshwater fish.

Silver carp Hypophthalmichthys molitrix, as a typical migration fish and widely distributed in China, played a major role in freshwater fisheries. However, with the dam construction, water pollution, over fishing and many other reasons, the natural resources of silver carp fell off sharply (Liu *et al*., 2005). In order to protect the resource of Silver carp, it is necessary to combine the function of ultrasonic telemetry into studying the movement, migration and habitat of it. Nevertheless, implantation of ultrasonic transmitters might have negative effects on the physiological status and behavior of fish. However, most of fishes with implanted transmitters are usually released relatively soon after tagging (Jepsen *et al*., 2000). In this case, the capture stress and surgical procedure may result in a higher mortality rates (Donaldson *et al*., 2011) and it is easier for telemetry equipment to detach (Bridger and Booth, 2003). Therefore, it is great significance to judge the health status of fish and determine the suitable releasing time

to eliminate the negative effects.

Analysis of biochemical parameters could help to identify target organs of injuries as well as the general health status of fish. The source of these parameters is the indicators responding to the environmental effects and can also serve as markers for surgical harm and recovery in fish (Luo *et al*., 2014). Environmental stress caused marked elevations in plasma glucose levels (Martin *et al*., 1998). The ALT, AST as well as cholesterol and chlorine can be use to establish the tissue damage. Total protein, globulin, albumin and triglyceride are indicative of nutritional status (Peres *et al*., 2014). Caputo *et al*. (2001) had comprehensively assessed stress response, tissue damage and nutritional status of largemouthbass surgically implanted with acoustic transmitters. It is advisable to assess the effects of surgical implantation of dummy ultrasonic transmitters through analyzing the biochemical parameters variation.

The objectives of our study were to determine the possible negative effects of surgical implantation of dummy ultrasonic transmitters on physiological response of silver carp and the duration of possible negative effects. 175 silver carps were used in this research to assess the possible effects of water environment and study the effects of surgical procedure and surgical implantation of dummy ultrasonic transmitter on biochemical parameters of the silver carp. We expected our results to provide basic information on the further study of the ultrasonic telemetry research, so as to apply the ultrasonic telemetry into freshwater fish.

Fish and Maintenance Conditions

Silver carps were obtained from the National Original Breeding Farm (NOBF) located in Jianli County, Hubei Province, China. Mean (± SE) body length and weight for fish were 627.5 ± 56.0 mm (range = 525-722 mm) and 8938.5 ± 2636.4 g (range = 4929-16123 g), respectively. All the fish are healthy and have no scars or broken scales on body and were placed in the circular tank (6 m in diameter and 1.5 m deep) for 14 days prior to surgery. The water was pumped from Laojianghe River, tanks received a 1/3 fresh water exchange per day. All the fish were exposed to a natural photoperiod and water temperature. The silver carps were fed twice a day at 10 am and at 5 pm and starved for 48 hours prior to surgery. The quantity of daily feeding accounted for 4.0% of the total weight of silver carps. The water quality was monitored throughout the experimental time. Average water temperature, in whole period, were 22.7 ± 0.9 ℃ (range from 22.2 to 24.1 ℃), pH, 7.44 ± 0.12 (range from 7.25 to 7.64), dissolved oxygen, 8.96 ± 0.17 mg/L (range from 8.73 to 9.23 mg/L), alkalinity 2.40 ± 0.06 mmol/L (range from 2.31 to 2.49 mmol/L) and hardness 136.5 ± 2.7 mg/L CaCO3 (range from 132.8 to 142.1 mg/L).

Experimental Design

Treatment began on June 3, 2011 and ended on July 5, 2011. 175 silver carps were assigned randomly into two groups: 1) Group one, 40 fish were used to assess the possible effects of water environment on fish; 2) Group two, 135 silver carps were divided randomly into three groups, handing but no tagging (control group), surgical procedure without implantation of dummy ultrasonic transmitter (sham group) and surgical implantation of dummy ultrasonic transmitter (surgery group), each group with 15 individuals were sampled 1 day, 7 days, and 14 days after tagging. The experiment was repeated thrice to reduce the randomness.

Surgical Procedure

The dummy ultrasonic transmitter (Shanghai Fishsonic Instrument Co., Ltd., Shanghai, China) measured 9 mm (diameter) × 29 mm (length) and weighted, on average, 4.7 g in air and 2.9 g in water. The transmitter's weight in air represented 0.05% of the mean body weight of the test fish. The surgical implantation of ultrasonic transmitters referred to the methods proposed by Moore *et al*. (1990), with the following modification: 1） Silver carps were anesthetized in 100 mg/L aerated buffered tricaine methanesulfonate (MS-222, Hangzhou animal medicine factory, Cen's Inc., China, Hangzhou) for about 5 min, then put them on V-shape operation platform, supplied with a maintenance anesthesia dose of MS-222 (40 mg/L). 2) After the routine measurement, a small incision (15-20 mm) was made parallel to the ventral midline, 10 mm off the trailing edge of pelvic fins and 20 cm away from the Celiac center. The incision depth was appropriate to just pierce their peritoneum. 3) the sterilized ultrasonic transmitters were put into the peritoneal cavity and sutured the wounds with the medical absorbable suture. 4) the wound was smeared with erythromycin ointment and fish were placed in an oxygenated tank (100 L) of fresh water for recovery.

Blood Collection

Fish from each experimental and control group were bled from the caudal vessels with a 5 ml needle syringe. The blood was placed in tubes at 4 ℃ containing the anticoagulant 1% dipotassium ethylenediamine tetra acetate (EDTA). The blood was centrifuged for 10 min at 4000 rpm. The plasma were preserved and stored at -10 ℃. Plasma samples were analyzed using an automated dry chemistry system (Abbott Architect c8000, RANDOX Laboratories Ltd., Ardmore, Crumlin, Co. Antrim, United Kingdom, BT29 4QY) for glucose, total protein, albumin, globulins, triglycerides, cholesterol, ALT and AST. The plasma chlorine levels were measured using a radiometer CMT 10 chloride titrator.

Data Analysis

SPSS 17.0 software was used for data processing. We calculated the mean values of all physiological variables and subjected them individually to analysis of variance (ANOVA). Duncan's multiple comparison procedure was used to compare the means of different groups. The significance level for all tests was $\alpha = 0.05$. All the values were expressed by mean ± SE.

Results

Effect on Water Environment

There was no significant difference between control groups at preoperative, 1 day, 7 days and 14 days after surgery in level of all blood biochemical parameter indexes (Table 1).

Effect on Glucose

There was significant increase in glucose level of sham and surgery groups 1 day after surgery ($p<0.01$), and it returned to normal in 7 days.

Effect on Total Protein, Albumin and Globulin

An increase in plasma total protein and globulin level was observed in sham and surgery groups in 1 day after surgery ($p<0.01$), whereas the albumin level significantly decreased ($p<0.05$). After 7 days, the level of total protein and globulin in sham and surgery groups began to decline but it was still significantly higher than that in control group ($p<0.01$). The albumin level continued to decrease ($p<0.01$). Both the albumin and globulin level returned to normal in 14 days.

Effect on Triglycerides, Cholesterol and Chlorine

There was no significant change in plasma triglycerides and cholesterol level of sham and surgery groups 1 day, 7 days and 14 days after surgery compared with control group. The level of plasma chlorine in sham and surgery groups significantly decreased 1 day after surgery ($p<0.01$), it began to rise in 7 days and returned to normal in 14 days.

Effect on ALT and AST

There was no significant change in plasma ALT level of sham and surgery groups 1 day, 7 days and 14 days after surgery compared with control group. The level of plasma AST in sham and surgery groups significantly increased 1 day after surgery ($p<0.01$), it began to decrease in 7 days and returned to normal in 14 days.

Table 1 The biochemical parameter values of silver carp in control group in preoperative, 1day, 7days and 14 days after surgery

Index	Preoperative	1 day	7 days	14 days
Glucose (mmol/L)	4.81±0.30	4.72±0.35	4.83±0.32	4.77±0.16
Total protein (g/L)	26.10±0.40	25.55±0.36	26.51±0.52	26.22±0.58
Albumin (g/L)	9.23±0.21	8.85±0.29	9.30±0.21	9.20±0.34
Globulin (g/L)	16.87±0.37	16.70±0.33	17.21±0.44	17.02±0.38
Triglyceride (mmol/L)	1.50±0.07	1.49±0.08	1.50±0.09	1.46±0.09
Cholesterol (mmol/L)	3.48±0.25	3.48±0.24	3.55±0.28	3.43±0.24
ALT (U/L)	66.20±3.26	60.60±4.32	59.60±3.73	59.30±3.42
AST (U/L))	133.20±5.88	135.90±8.15	135.80±10.30	134.30±11.95
Chlorine(mmol/L)	97.90±2.33	96.81±2.36	97.75±2.33	97.68±2.16

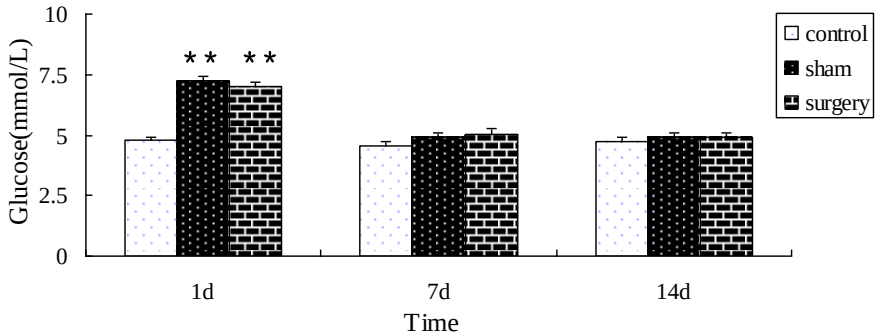

Figure 1. The level of plasma glucose in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

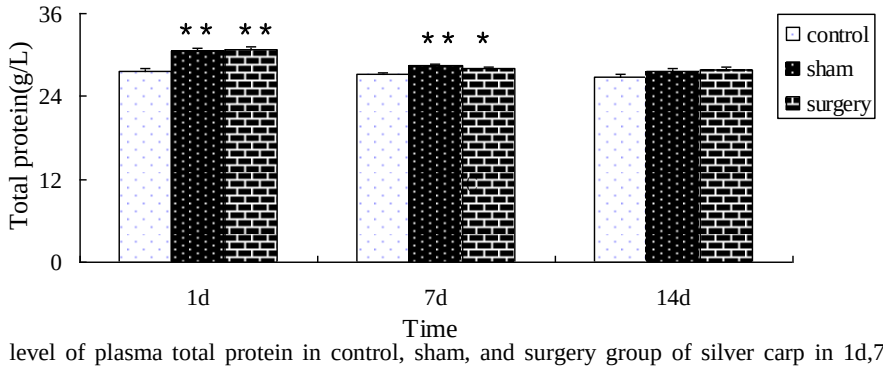

Figure 2. The level of plasma total protein in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

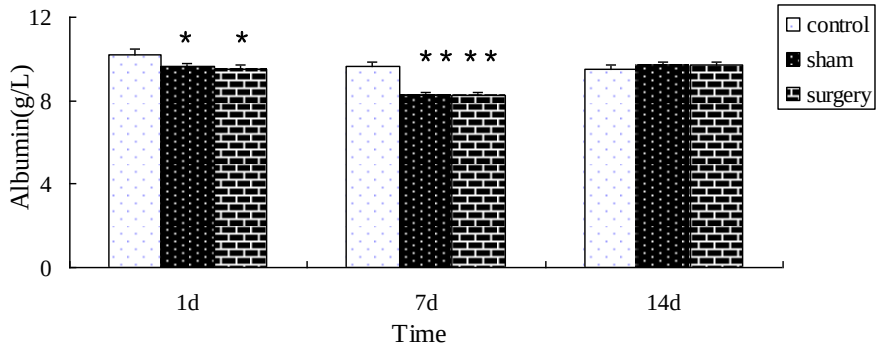

Figure 3. The level of plasma albumin in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

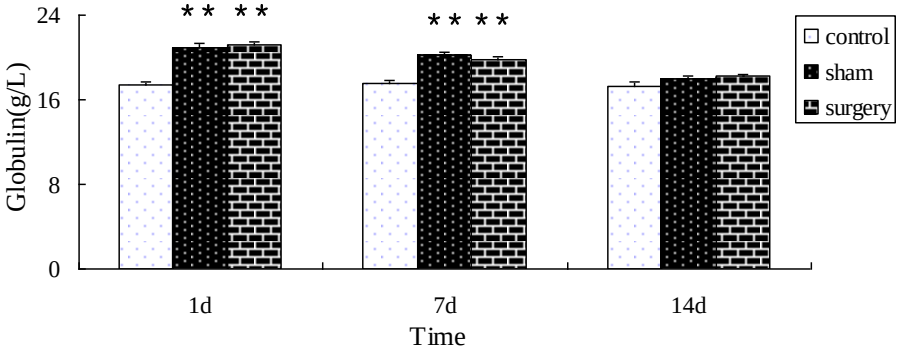

Figure 4. The level of plasma globulin in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

Discussion

There was no significant difference between control groups at preoperative, 1 day, 7 days and 14 days after surgery in level of all blood biochemical parameter indexes. The fish were in good conditions during the experimental process. All incisions of the silver carps had been healed in the 14 d after the surgery, and all tested individuals were survived in this experiment. Five sutures of the silver carps did not fall off. The suture off rate was 94.44%. Two silver carps discharged launcher in the 7 days after the surgery through the incision. Emitter discharge rate was 4.44%.

Plasma glucose is regulated by complex interactions of hormones such as glucagons and cortisol (Shweta, 2007). The plasma glucose level is widely used as the primary indicator of stress in fish (Wedemeyer *et al*., 1990; Barton and Iwama, 1991). The plasma glucose in high level can help to meet the energy demand of fish in the stress process (Qiang *et al*., 2012), and this was apparent in the present study. The plasma glucose level of sham and surgery groups increased significantly 1 day after surgery indicated

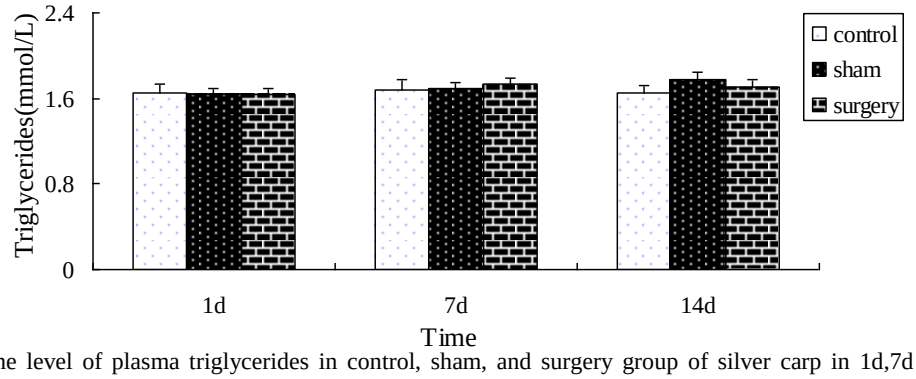

Figure 5. The level of plasma triglycerides in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

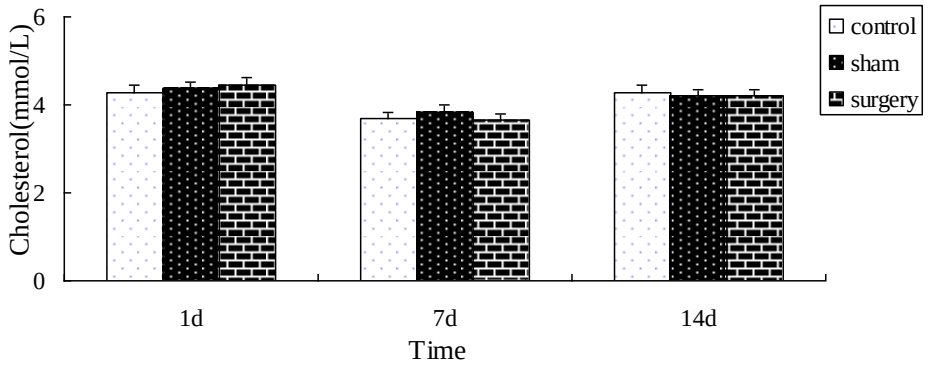

Figure 6. The level of plasma cholesterol in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

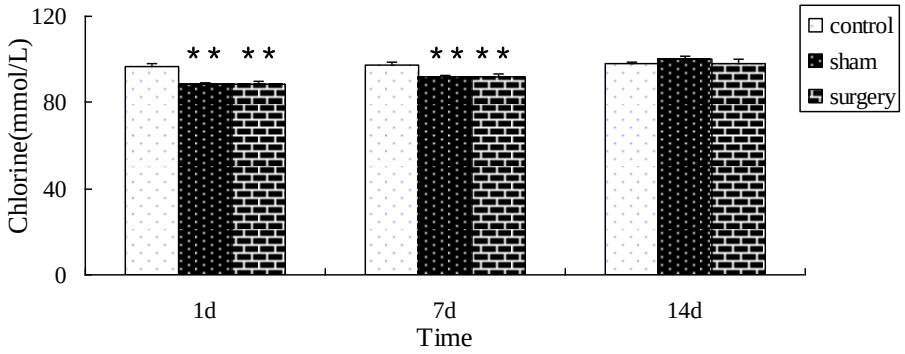

Figure 7. The level of plasma chlorine in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

that silver carps generated a stress response after the surgical procedure. The result was different from bighead carp whose plasma glucose level increase 3 hours after surgery and recovered in 24 hours (Luo *et al*., 2014). Discrepancies between these results may due to the traits of the species. As is well known, silver carps were active while bighead carps were meek and sluggish. More options would add the probability of further damaged of the wound and it would prolong the stress response time.

Plasma protein levels are often associated with fish nutritional and physiological status. Globulins constitute a number of heterogeneous proteins, including coagulation factors, transport protein, mediators of inflammation and immunoglobulins (Haschek *et al*., 2010). In our study, plasma globulin level increased significantly in 1 day after the surgery and it last for 7 days. The increase in the globulin level might be due to increase in C-reactive protein levels. The surgical procedure initially caused incision

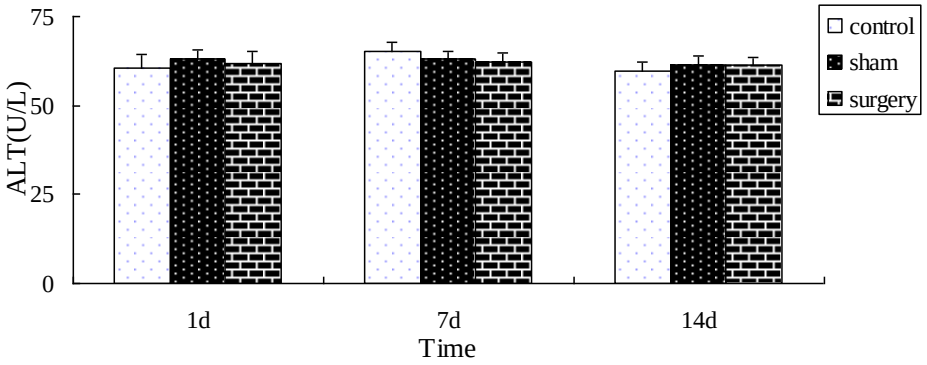

Figure 8. The level of plasma ALT in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

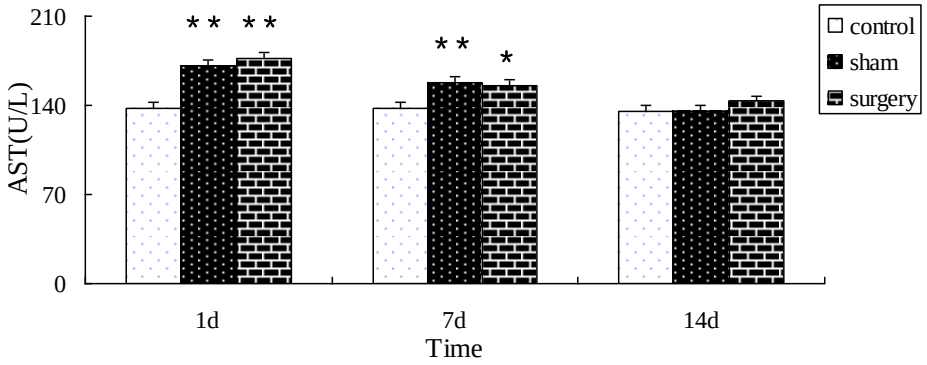

Figure 9. The level of plasma AST in control, sham, and surgery group of silver carp in 1d,7d and 14d after surgery(N=15). Significance level is indicated with asterisks (*P＜0.05 ; **P＜0.01).

damage and bacterial infection, and the act of C-reactive protein as an autologous protective mechanism against autoimmunity through binding to damaged tissue surfaces via phosphorycholine and glycoprotein and enhanced phagocytosis (Gopal *et al*., 1997). The decreased in the level of globulin in sham and surgery groups indicated that silver carps were not in good nutritional status. And this was similar to the results of Qian Yunxia' research that starvation induced a significant reduction in plasma protein levels. Indeed, under stress conditions, altered plasma total protein levels often occur as consequence of amino oxidation or peripheral proteolysis.

Cholesterol and triglyceride are derived from ingested food and endogenous synthesis, particularly by the liver (Haschek *et al*., 2010). In the present study, there was no significant change in plasma triglycerides and cholesterol level of sham and surgery groups 1 day, 7 days and 14 days after surgery compared with control group. It was similar to some other research which showed that plasma cholesterol and triglyceride levels are not responsive to acute stress (Ruane *et al*., 2001; Li *et al*., 2011).

Plasma enzyme activity may also provide important information on the functional state of different organs, as intracellular and plasma enzymatic concentrations are usually proportional (Helena Petes, 2014). Plasma ALT activity is a good indicator of hepatic damages (Mitchell *et al*., 1980), since the concentration of this enzyme in hepatic tissue is higher than the other tissues and cellular damages lead to the enzyme leakage. AST is present in many tissues, so that, it is not a specific indicator of liver damage, but its elevation shows there is a cellular damage in the body. In our research, there was no significant change in the level of plasma ALT after surgery, but the level of plasma AST significantly increased in 1 day and 7 days. It is obviously that the surgical procedure caused the tissue damage seriously. Nevertheless, it did not hurt the liver tissue. It is to some extent in accordance with the result of the alteration of cholesterol and triglyceride. Both of them are chylomicrons' structure, which are eliminated by the hepatobiliary system. Any hepatobiliary disorder may lead to decrease in cholesterol and triglyceride levels. The decreased in albumin level was not due to the dysfunction of liver that lead to the protein synthesis failure. It was due to the blood lost during the surgical process and over-consuming of protein in repair of the damaged tissue.

Chlorine is one of the most abundant ions in fish blood. In our research, the level of plasma chlorine in

sham and surgery groups significantly decreased 1 day after surgery and last for at least 7 days. Although there are many reference studies pointing out that fish gill is the most important site for osmoreglation, particularly regulation of sodium and chloride. The decreased in plasma chlorine level might result from structural or functional damages of kidney, the dysfunction of reabsorption would result in loss of a large amount of plasma chlorine. This was in accordance with the research of silver carp in septicemia.

Conclusion

The blood biochemical parameters can be used to evaluate the influence of surgical implantation of ultrasonic transmitters on silver carp. The technology of ultrasonic transmitters can be used in the further study of the movement, migration and habitat of the silver carp, but the surgical procedure did have some negative effects on them. The main results were the stress response, tissue damage and the structural or functional damages of kidney, but the function of the liver was in normal. All the blood biochemical parameters returned to normal in 14 days after surgery, so we recommend that all the silver carp should be cultured at least for 14 days after surgery before the further research.

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