Blood Sampling from the Cuvierian Duct of Red Sea Bream (*Chrysophrys major*) for Hematology and Blood Biochemistry in Toxicological Studies

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ABSTRACT. To investigate a suitable blood sampling method for hematology and blood biochemistry in fish toxicology, we compared two methods, blood sampling from the Cuvierian duct with a syringe (CD) and caudal vein puncture (CVP) in red sea bream, *Chrysophrys major*. The CD method did not require special skill because the Cuvierian duct was externally visible. This method allowed us to obtain blood directly from the vessel without disrupting other tissues. The maximum obtainable blood volume withdrawn by CD method was the same as that by CVP method. Clots were occasionally observed in the blood sampled by CVP method, but not by CD method. Activities of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were much higher in the plasma obtained by CVP than by the CD method. The activity of glutamate oxaloacetate dehydrogenase (GOT) in CVP was also higher, but to a lesser extent. The values of LDH, CPK and GOT from CVP were much more variable. The correlation coefficients between these values from CVP were higher. Because CPK and LDH are highly concentrated in the muscle, the concomitant elevation of CPK, LDH and GOT activities from CVP could be caused by a leak of these enzymes from muscle cells disrupted by the inserted needle. No significant differences existed in total blood cells, hemoglobin and other biochemical parameters examined between blood samples obtained from either method. Thus the CD method is considered to be a practical and reliable method for blood biochemistry and hematology in fish toxicology.—**KEY WORDS:** blood sampling, Cuvierian duct, enzyme leakage, fish toxicology, serum enzyme.

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The determination of hematological and blood biochemical parameters in fish toxicity studies is a useful tool as it is in mammalian studies. For this purpose, enough volume of intact blood should be obtained quickly without special technique.

For fish toxicity testing, we sometimes have to use small fish because of restrictions of space, limited amount of chemicals and some technical difficulties for treatment of the discharge of chemicals. Blood sampling from fish, especially from small ones, is difficult since the total blood volume in fish is less than that of mammals (about 2–7 ml/100 g of body weight). The blood of fish is also very coagulable. Some techniques have been devised for blood sampling from fish: caudal vein puncture (CVP), cardiac puncture, cannulation to the dorsal aorta, and the

severance of the caudal peduncle [2]. The blood vessels for conventional blood sampling are located deep in the fish body.

The method of blood withdrawal affects the results of hematological and blood biochemical analysis. Contamination of enzymes and other constituents from surrounding tissues is considered to be the main source of error [4]. Various investigators [1, 3, 5] reported that cardiac sampling would be the only feasible way for the determination of plasma enzymes because of less contamination by tissue fluid than with other methods. Even with this method, the same authors suspected that an injury to the heart muscle by puncture would cause leakage of tissue components into the sample. Cardiac sampling requires special techniques and experience. The maximum obtainable blood volume from cardiac punc-

Fig. 1. Cuvierian duct site for blood sampling from red sea bream.

ture is less than that from CVP, and cardiac puncture is very difficult to use with small fish.

Sano [7] and Lied *et al.* [6] described a technique for blood sampling from the Cuvierian duct in rainbow trout. However, blood samples from the Cuvierian duct have not been used for hematological and blood biochemical examination in toxicological studies.

To obtain a suitable method of blood sampling in fish toxicological studies, we developed a modified sampling method from the Cuvierian duct (CD). The blood withdrawn by the CD method were compared with that by CVP on hematological and blood biochemical parameters. Red sea bream was chosen as a candidate fish because it is one of the most economically important in Japan.

MATERIALS AND METHODS

Fish: Red sea bream, *Chrysophrys major*, were purchased from Shizuoka Thermal Effluent Utilization Research Center (Shizuoka, Japan). The mean weight was 54 ± 10 g (mean $\pm$ SD) at the time of use. Fish were kept in

circular polyethylene tanks of 400 l capacity. Holding tanks were supplied with a continuous flow of natural sea water which was recycled after filtration. The water temperature was maintained at $22 \pm 1^\circ\text{C}$. Fish were acclimatized to laboratory conditions for 10 days prior to blood sampling.

Blood collection: Fish were anesthetized in a 1:5000 w/v solution of tricain methane sulfonate (MS-222). Blood was withdrawn using an 1-ml tuberculin-syringe equipped with a 27 G hypodermic needle. Tuberculin-syringes and test tubes were previously heparinized and dried under an overnight vacuum at room temperature. CVP method was done in a conventional manner. The CD method was as follows. After incision of the epidermis which covered a clavicle and the supporting tissues, the needle was inserted into the exposed Cuvierian duct in parallel with the clavicle (Fig. 1).

Hematology and blood biochemistry: The number of total blood cells (TBC) was measured with a Coulter Counter® Model D (Coulter Electronics, Inc., Hialeah FL). Hemoglobin values (Hb) were determined by the cyanmethemoglobin method using a Shimazu

Table 1. Methods used for determination of blood biochemical parameters

Parameter	Method
LDH	NADH measurement at 340 nm ^{a)}
CPK	NADPH measurement at 340 nm ^{c)}
GOT	NADH measurement at 340 nm ^{a)}
ALP	Modified Bessay-Lowry ^{a)}
GPT	NADH measurement at 340 nm ^{a)}
γ -GTP	Modified Orłowski ^{a)}
Glucose	Glucose oxidase—peroxidase ^{a)}
T. chol	Cholesterol oxidase—peroxidase ^{a)}
TG	Glycerol kinase—glycerophosphate oxidase—peroxidase ^{b)}
TP	Biuret ^{a)}
UA	Uricase—peroxidase ^{b)}
Ca	o-Cresolphthalein complexone ^{a)}
Pi	Modified Fiske-Subbarow ^{a)}

Standard method using the commercial kits purchased from

a) Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan).

b) Sanko Junyaku Co., Ltd. (Tokyo, Japan).

c) Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Table 2. Blood constituents of red sea bream from two sampling methods

Parameters	unit	Cuvierian Duct			Caudal Vein		
		mean $\pm$ S.D.	CV ^{a)}	N ^{b)}	mean $\pm$ S.D.	CV	N
LDH	U/l	46 $\pm$ 15 ^{c)}	33	19	216 $\pm$ 163	75	10
CPK	U/l	34 $\pm$ 20 ^{c)}	59	18	205 $\pm$ 128	62	10
GOT	U/l	20 $\pm$ 7	35	19	29 $\pm$ 15	52	10
ALP	U/l	160 $\pm$ 76	48	19	147 $\pm$ 85	58	10
GPT	U/l	14 $\pm$ 11	78	19	12 $\pm$ 6	50	10
γ -GTP	U/l	21.2 $\pm$ 3.3	16	19	23.2 $\pm$ 5.2	22	10
TBC	10 ⁶ /mm ³	3.3 $\pm$ 0.7	20	18	3.2 $\pm$ 0.7	21	10
Hb	g/dl	9.6 $\pm$ 0.8	8	18	9.6 $\pm$ 0.9	9	10
Glucose	mg/dl	136 $\pm$ 30	22	19	135 $\pm$ 79	59	10
T. chol	mg/dl	426 $\pm$ 74	17	19	438 $\pm$ 55	13	10
TG	mg/dl	520 $\pm$ 304	58	19	574 $\pm$ 351	61	10
TP	g/dl	4.4 $\pm$ 0.3	8	19	4.3 $\pm$ 0.5	12	10
UA	mg/dl	0.9 $\pm$ 0.4	44	18	0.8 $\pm$ 0.2	25	10
Ca	mg/dl	14.0 $\pm$ 0.9	6	19	14.5 $\pm$ 1.3	9	10
Pi	mg/dl	10.9 $\pm$ 2.1	19	19	10.7 $\pm$ 2.0	19	10

a) Coefficient of variation in percent.

b) Number of fish examined.

c) Significantly different from CVP ($p < 0.01$).

spectrophotometer Model UV-300 (Shimazu Corporation, Kyoto). The remaining blood was centrifuged to separate plasma. The resultant plasma was analyzed with a blood

biochemical autoanalyzer, Hitachi 726 (Hitachi Ltd., Tokyo) for the following parameters: lactate dehydrogenase (LDH), creatine phosphokinase (CPK), alkaline phosphatase

Table 3. Correlation coefficients between enzyme activities in plasma of red sea bream from two sampling methods

Enzymes correlated	Cuvierian Duct		Caudal Vein	
	r ^{a)}	N ^{b)}	r	N
CPK vs LDH	0.71	10	0.86	18
LDH vs GOT	0.73	10	0.95	19
CPK vs GOT	0.57	10	0.77	18

a) Correlation coefficient.

b) Number of fish used in the correlation.

(ALP), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), γ -glutamyltranspeptidase (γ -GTP), blood glucose (Glucose), total cholesterol (T. chol), triglyceride (TG), total protein (TP), uric acid (UA), calcium (Ca) and inorganic phosphorus (Pi). The methods for determining plasma constituents are tabulated in Table 1. Differences between values for the blood parameters measured were tested for statistical significance using Aspin-Welch *t*-test.

RESULTS

The same blood volume (ca 0.7 ml) was obtained with both methods. The blood sample withdrawn by CVP occasionally contained blood clots, but that of CD method did not. Activities of LDH and CPK in the plasma sampled by CVP were significantly higher (5–6 times) than those obtained by the CD method (Table 2). GOT activity was slightly higher in plasma sampled by CVP than by the CD method, although no statistical significance was obtained. Individual activities of LDH, CPK and GOT in plasma sampled by CVP were more variable than with the CD method, as shown by larger coefficients of variation. The correlation coefficients among activities of LDH, CPK and GOT were higher in plasma obtained by CVP than by the CD method (Table 3). Other biochemical parameters measured were unaffected by the sampling methods. No significant differences existed in TBC and Hb between the two

methods.

DISCUSSION

The Cuvierian duct is the only vessel externally visible in fish, and a needle can be easily inserted without special techniques. This method enable even an inexperienced person to withdraw blood from many fish within a short time period, and the method did not cause damage to other tissues except for the thin-walled Cuvierian duct itself. The results indicated that the activities of LDH, CPK and GOT were low and considerably stable in plasma sampled by the CD method.

Activities of LDH, CPK and GOT were elevated and variable in plasma obtained with the CVP method although the GOT elevation was not statistically significant. Correlation coefficients between activities in plasma prepared by CVP were higher than those by the CD method. The skeletal muscle of fish contains large amount of LDH and CPK, and it also contains GOT but to a lesser extent [1]. The lower increase of GOT in plasma obtained by CVP may be due to its relatively low activity in the muscle. The concomitant increase of LDH, CPK and GOT activities in plasma sampled by CVP might have resulted from contamination by enzymes from disrupted muscle cells caused by insertion of the needle.

Hille and Pöllnitz [3], and Ikeda and Ozaki [5] analyzed blood samples obtained from two different methods, cardiac puncture and severing the caudal peduncle in rainbow trout. Gaudet *et al.* [1] compared plasma enzymes of rainbow trout sampled by cardiac puncture and CVP. All these authors reported that cardiac sampling was the only suitable way for enzyme determinations. However, they suspected that accidental enzyme leakage caused by heart puncture would not be excluded because the heart muscle has high activities of CPK, GOT and LDH.

The present studies demonstrated that the

CD method was the only method eliminating contamination of plasma by leaked enzymes employed so far. Many other species of fish have the Cuvierian ducts with similar shape and location. Thus, the CD method could be applied to various species of small fish. Therefore the CD method is considered to be a suitable and practical method for hematology and blood biochemistry in fish toxicity studies.

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要 約

毒性試験における血液学および血液生化学的検査のためのマダイの Cuvier 氏管からの採血法：池田茂美子・津田修治・倉若欣司・深尾隆三・白須泰彦（残留農薬研究所）——毒性試験における血液学および血液生化学的検査に適した採血法を検討する目的で、マダイにおいて Cuvier 氏管採血法（CD）と汎用される尾静脈穿刺法（CVP）とを比較検討し、CD が有用であることが示された。Cuvie 氏管は鰓蓋を押し上げると直視できるので、熟練を要せず、周辺組織に損傷を与えることなく採血が可能であった。CD による採血量は CVP と同程度であったが、ときに CVP で生ずる血塊形成は CD では認められなかった。CD によるものにくらべて、CVP によるサンプルの測定値は乳酸脱水素酵素（LDH）とクレアチンリン酸キナーゼ（CPK）活性は 5～6 倍、グルタミン酸オキザロ酢酸トランスアミナーゼ（GOT）活性は 1.5 倍であった。CVP ではこれら 3 種の酵素活性値の変動が大きく、かつ 3 者間の相関は CD にくらべて非常に強かった。CPK と LDH は筋肉中で高値であることから、CVP では穿刺により損傷された筋肉からの酵素漏出があることが示唆された。その他の検査値には両法に差は認められなかった。