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Effects of the Vibration Stress on Cortisol and Hematological Characteristics in Soft-shelled Turtle, *Pelodiscus sinensis*

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진동 스트레스에 따른 자라, *Pelodiscus sinensis*의 코티졸 및 혈액학적 특성

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ABSTRACT : We examined the effects of the vibration stress on cortisol secretion and hematological characteristics in soft-shelled turtle, *Pelodiscus sinensis*. For the stressed group vibration of 45~78 dB(V) from electric vibrator applied for 30 min with 2-h intervals during daytime (08:00~18:00) up to 28 days. Using the blood samples collected from ten turtles held once a week after vibration stress, we measured hematocrit, hemoglobin, red blood cells, cortisol, glucose, lactic acid, osmolality, Na^+, K^+, Cl^-, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The results have showed that *P. sinensis* received vibration stress exhibit the 'typical' stress-induced physiological responses (cortisol, glucose, lactic acid, osmolality, ions, hematocrit and hemoglobin) induced by vibration stress. Our data suggested that chronic vibration stress caused substantial stress in the animal, and in particular, the persisting elevated levels of AST and ALT would be highly correlated with the adverse effects of the stress. The high hematological characteristics during entire experimental period showed that the *P. sinensis* could not adapt to chronic stimuli provoked by vibration stress.

Key words : Soft-shelled turtle, *Pelodiscus sinensis*, Vibration stress, Hematological characteristic, Cortisol.

INTRODUCTION

The soft-shelled turtle, *Pelodiscus sinensis* is a reptile that belongs to the order Testudinata, and members of the family Trionychidae are frequently used as medicines, gourmet and health foods in Korea and China. In its natural setting, this animal is an omnivore and eats water plants, insects, and small fish. It is nocturnal and its habitats include rivers, ponds, swamps, and water reservoirs with sandy mud (Kim, 1998). Its natural habitat and spawning grounds are being destroyed in Korea due to urbanization and particularly by gravel removal. Industrial and residential wastewater further reduces its available habitat. In the 1990's, its population was increased by seedling production and aquaculture. In a natural setting the *P. sinensis* is a secretive animal and very sensitive to noise and vibration (Lee et al., 2007). The *P. sinensis* farms are best located

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where the reptile is unlikely to be affected by environmental stresses; however, some are exposured to noise and vibration.

Reports have been issued on the hatching, seedling production, and rearing of baby *P. sinensis* (Kim, 1998) and on stress response comparison after vitamin C and E ingestion (Zhou et al., 2004). Investigations of response to stress have been mainly conducted on hormone secretion due to handling and capture (Gregory et al., 1996; Cash et al., 1997; Mahmoud & Licht, 1997; Gregory & Schmid, 2001; Jessop et al., 2002). However, data on the response of *P. sinensis* to environmental stresses are rare, and in particular no reference data is available on physiological response in terms of blood components.

In the present study, we examined the effects of the vibration stress on cortisol and hematological characteristics in *P. sinensis*, which it is likely to be exposed to during the farming process.

MATERIALS AND METHODS

Experimental animals were 3～4 year-old *P. sinensis* with a 16.8±1.8 ㎝ carapace length and average weight 0.7±0.2 ㎏. They were purchased from a farm and habituated in a lab aquarium tank for 2 weeks before use in the study. The *P. sinensis* in stressed group (*n*=20) were placed in a tank (2 ton, FRP, raceway style), and an electric vibrator was attached to one end side of tank outer. For the stressed group vibration of 45～78 dB(V) from electric vibrator applied for 30 min with 2-h intervals during daytime (08:00～18:00) up to 28 days. In order to exclude other stresses than vibration, without other disturbances, and the water temperature was automatically regulated at 25℃ using a temperature control system. The *P. sinensis* in the non stressed group (*n*=20) were maintained under the same environmental conditions excluding vibration for 28 days.

Blood was sampled during 1 min without anesthesia from the neck blood vessel complex using heparinized syringes at day 0 (before start of the experiment, BE), 7, 14, 21, and 28 days after vibration stress.

Hematocrit, red blood cells, and hemoglobin were analyzed immediately using an automatic blood analyzer (Excell 500, USA). Blood samples were kept in 2-㎖ vacuum containers treated with sodium fluoride/potassium oxalate (Vacutainer, UK) and in 1.5-㎖ polypropylene microcentrifuge tubes held on ice for less than 5 min before centrifugation at 5,600 *g* for 5 min. Plasma was then collected and stored in a deep freezer (CLN-500 UW Nihon Freezer; Nihon Co., Japan) at －70℃ until analysis.

Plasma cortisol concentrations were determined in 50-㎕ samples using radioimmunoassay kits (Coat-A-Count TKCO Cortisol RIA Kit; DPC, USA). Mixtures of samples in 100 ㎖ of antiserum were incubated for 45 min at 37℃, and then 1,000 ㎖ of separation reagent was added. The mixtures were placed in a refrigerator at 4℃ for 15 min, then centrifuged at 1,200 g for 15 min. The supernatant was assayed for gamma radiation using an automatic gamma counter (Cobra Ⅱ; Packard Co., USA). Glucose, lactic acid, AST (aspartate aminotransferase), ALT (alanine aminotransferase), Na^+, K^+, Cl^-, and total protein were analyzed using an automatic chemistry analyzer (Hitachi 7180, Hitachi, Japan). Osmolality was determined using a micro-osmometer (Fiske 210, Fiske, USA).

Experiment was performed in triplicate and results are reported as means±SD (*n*=4) unless otherwise stated. Data were analyzed by a one-way ANOVA in the SPSS (Statistical Package for Social Sciences) statistical package. Means were separated by using Duncan's multiple range test and were considered significantly different if $P<0.05$.

RESULTS AND DISCUSSION

Most vertebrates exhibit conspicuous physiological responses when exposed to ecological or environmental stressors (Wingfield et al., 1998). Considerable variations in plasma corticosterone levels can be induced by both internal and external factors (Creel et al., 1997; Dunlap & Wingfield,

1995; Mara & Holberton, 1998; Romero, 2002).

Variations in environmental factors, such as temperature, rainfall, food availability, humidity, and habitat quality, are known to influence basal and stress-induced plasma corticosterone concentrations in natural populations (Kitaysky et al., 1999; Mara & Holberton, 1998; Wingfield & Ramenofsky, 1999). In addition, variations in adrenocortical function among individuals can arise due to overall body condition, disease status, age, sex, and social status (Creel et al., 1997; Dunlap & Wingfield, 1995; Grassman & Hess, 1992; Kitaysky et al., 1999; Knapp & Moore, 1996; 1997). Thus, corticosterone plasma profile is potentially a valuable hormonal index of stress and the internal and external factors that influence the physiological functioning of animals at the individual and population level.

In this study, the before-stress (basal) cortisol found in this study (3.7 ng/㎖) was lower than the values reported previously for the *P. sinensis* (Zuou et al., 2004), but its was similar to the values found also by other authors working with red-eared slider turtles, *Trachemys scripta* (Cash et al., 1997), ridley sea turtles, *Lepidochelys kempii* (Gregory & Schmid, 2001), and green turtles, *Chelonia mydas* (Jessop et al., 2002).

Plasma cortisol and glucose concentration in the stressed group increased significantly ($P<0.05$) during the experiment period (Fig. 1). Cortisol and glucose at equivalent days from 7 to 28 days were significantly affected by vibration stress.

Cortisol and glucose were found to increase in a stress dependent manner. Barton & Iwama (1991) reported that these increases are the result of metabolic responses and that glucose is produced as a result of gluconeogenesis due to the action of cortisol produced in response to stress. Since we found increased levels of plasma factors in our study, we conclude that stressed and non-stressed were stressed.

Long-term studies on chronic physiologic responses to vibration are rare. Some short-term studies have reported increased cortisol levels related to stress in turtles. This

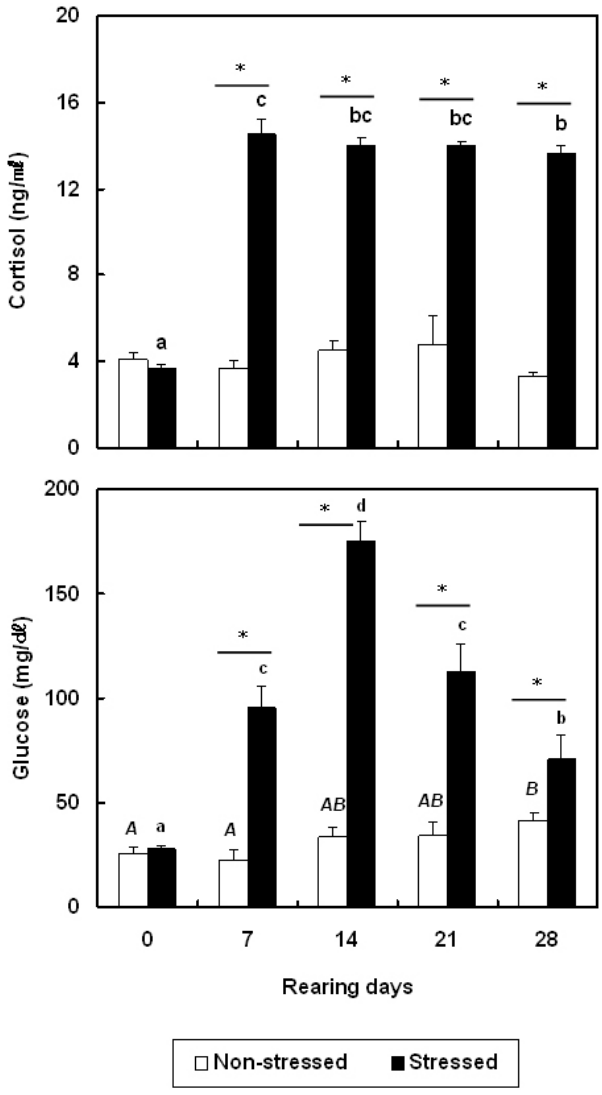

Fig. 1. Effects of the vibration stress on plasma cortisol and glucose levers of soft-shelled turtle, *P. sinensis*. Values are means ± SD (n=4) for experiments run on two occasions. Shared alphabetic letters on shaded bars indicate lack of significant difference (Duncan's multiple range test $P>0.05$). * $P<0.05$ indicates significance between low and high densities at that time point.

increased was reported to be due to capture in sea turtles, *Caretta caretta* (Gergory et al., 1996), red-eared slider turtles (Cash et al., 1997), green turtles (Jessop et al., 2002), and hawksbill turtles, *Eretmochelys imbricata* (Jessop et al., 2004). However, we believe that long-term exposure to

vibration affects the animals more seriously than capture, as cortisol levels remained high for 28 days after stress. Moreover, increased glucose levels were reported to be induced by handling in sea turtles (Gregory & Schmid, 2001) and three-toed box turtles, *Terrapene carolina triunguis* due to temperature increases (Sturbaum & Bergman, 1981). Thus, we believe that the high levels of cortisol and glucose observed in soft-shelled turtles indicated that animals were stressed.

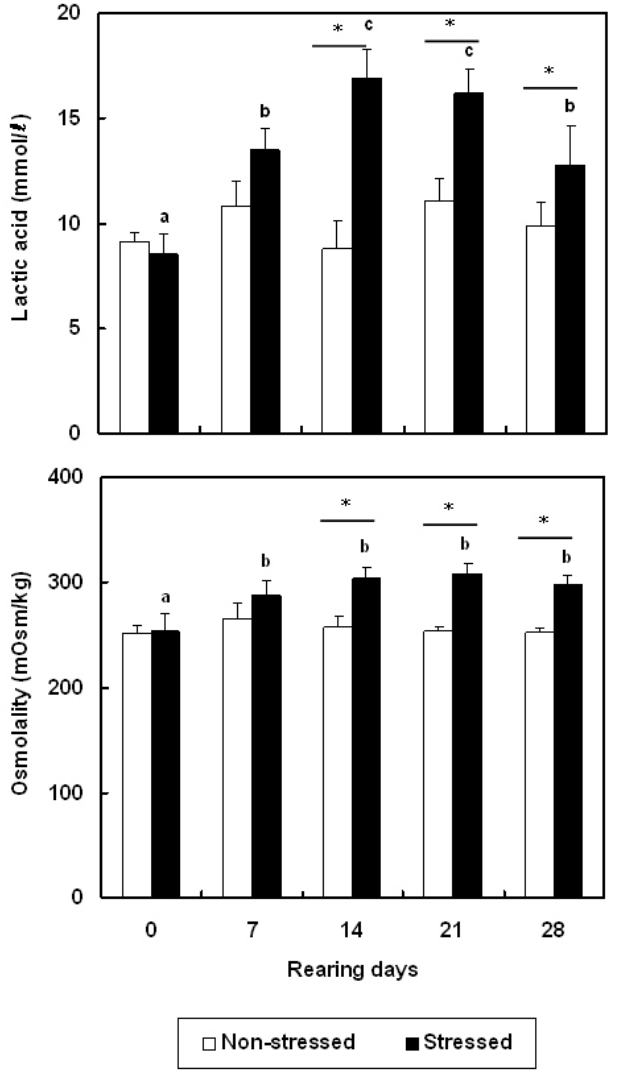

Fig. 2. Effects of the vibration stress on plasma lactic acid and osmolality of soft-shelled turtle, *P. sinensis*. Values are means ± SD (n=4) for experiments run on two occasions. Shared alphabetic letters on shaded bars indicate lack of significant difference (Duncan's multiple range test $P>0.05$). * $P<0.05$ indicates significance between low and high densities at that time point.

Plasma cortisol, glucose, lactic acid and osmolality in the stressed group increased significantly ($P<0.05$) during the experiment period (Fig. 1 and 2). Lactic acid and osmolality at equivalent days from 7 to 28 days were significantly affected by vibration stress.

We suggest that lactic acid levels are related to the habitation of *P. sinensis*. The *P. sinensis* is a nocturnal reptile, and it is believed that lactic acid increased because turtles in the stressed group moved constantly, whereas animals in the non-stressed group largely remained stationary during the day. Lactate acid may be a result of a higher metabolic rate due to stress and moving when compared to stress group from non-stressed group.

The stressed group also showed higher levels of osmolality and ion (Na^+, Cl^- and K^+) disruption than the non-stressed group. *P. sinensis* tend to maintain homeostasis, i.e., pH, glucose, and ions and osmolality. In general, fish achieve osmoregulation and respire through the gills, gastric tract, and kidney, and maintain homeostasis by controlling water and ion levels. The *P. sinensis* lives on both water and land, and breathes using its lungs and skin. Fish achieve osmoregulation by absorbing ions (Na^+, Cl^-, and K^+) from the gastric tract wall and discarding them through the kidney (Kirsch & Meister, 1982). We suggested that the *P. sinensis* has a similar osmoregulation mechanism.

In plasma AST and ALT, the stressed group showed a significant ($P<0.05$) increase at 28 days than BE (Table 1). AST and ALT are aminotransferases that are distributed in liver and spleen. Levels are low when animals are healthy but increase when tissue necrosis is present or an animal becomes sick. Davis & Parker (1990) reported that hemodynamic indices of oxygen carrying ability are increase by stress. In the present study, AST and ALT levels in the stressed group were significantly elevated by chronic vibration stress, suggesting that vibration places physiological burdens on liver and spleen. AST and ALT levels

Table 1. Effects of the vibration stress on hematological characteristics of soft-shelled turtle, *P. sinensis*

	Rearing days	Experimental groups	
		Non-stressed	Stressed
Na^+ (mEq/ℓ)	0	108.5± 6.4	117.5± 9.2^{c}
	7	107.5± 4.9	112.0± 1.8^{bc}
	14	109.0± 1.4	111.8± 4.3^{bc}
	21	109.0± 2.8	106.0± 6.9^{b}
	28	106.0± 2.8	85.5± 0.7^{a,*}
K^+ (mEq/ℓ)	0	2.4± 0.1	1.9± 0.3^{a}
	7	2.3± 0.2	2.7± 0.2^{b}
	14	2.6± 0.1	3.0± 0.4^{b}
	21	2.9± 0.2	2.9± 0.2^{b}
	28	3.1± 0.7	22.1± 0.8^{c,*}
Cl^- (mEq/ℓ)	0	76.0± 4.2	84.5±12.0^{c}
	7	74.5± 2.1	79.8± 1.7^{bc}
	14	78.5± 6.4	78.0± 2.8^{bc}
	21	78.5± 4.9	69.8± 6.4^{b}
	28	72.5± 2.1	60.0± 0.1^{a,*}
AST (IU/ℓ)	0	110.7±10.6	102.3± 5.8^{a}
	7	112.5± 6.0	330.1±24.3^{b,*}
	14	125.9±10.3	311.8± 8.1^{b,*}
	21	138.4±15.3	248.5±25.0^{b,*}
	28	133.7± 6.4	559.8±98.3^{c,*}
ALT (IU/ℓ)	0	5.0± 1.4^{a}	4.0± 0.7^{a}
	7	3.5± 0.7^{a}	4.0± 0.8^{a}
	14	6.0± 1.4^{ab}	4.5± 1.0^{a}
	21	9.8± 2.5^{b}	6.0± 2.6^{a}
	28	5.1± 1.1^{a}	43.2± 8.0^{b,*}
Hematocrit (%)	0	12.8± 0.6	13.0± 0.6^{a}
	7	12.6± 0.8	13.9± 1.2^{ab}
	14	13.5± 0.7	15.9± 0.2^{b,*}
	21	12.5± 0.2	15.7± 0.7^{b,*}
	28	12.9± 1.2	14.5± 0.7^{ab,*}
Hemoglobin (g/dℓ)	0	12.1± 0.6	12.5± 0.6^{b}
	7	11.0± 1.4	10.1± 1.3^{ab}
	14	10.6± 0.8	7.0± 1.4^{a,*}
	21	12.5± 0.7	7.6± 0.6^{a,*}
	28	12.3± 1.5	7.7± 1.8^{a,*}
Red blood cell ($\times10^6$ cell/$\mu\ell$)	0	6.5± 0.4	6.0± 0.4
	7	6.6± 0.8	6.4± 0.4
	14	5.5± 0.7	6.0± 0.6
	21	6.0± 0.2	7.2± 0.8
	28	5.9± 0.6	7.3± 0.6

Values are means ± SD (n=4) for experiments run on two occasions. Means sharing the same superscripted letter are not significantly different (Duncan's multiple range test $P>0.05$). * indicates significant differences between groups at equivalent days ($P<0.05$).

were increased by 70% and 112%, respectively, after exposing three-toed box turtles temperature stress (Sturbaum & Bergman, 1981), which supports the result of this study.

Hemodynamic indices such as hematocrit, red blood cells, and hemoglobin represent oxygen carrying ability. Davis & Parker (1990) reported that stress increases hematocrit, red blood cell, and hemoglobin levels. These indices may depend on acute and chronic stress. Sturbaum & Bergman (1981) reported that levels of hematocrit, red blood cells, and WBCs were reduced by temperature stress. However, we found increased hematocrit levels and reduced hemoglobin levels. The difference is probably due to our application of chronic stress.

In conclusion, our results have shown that *P. sinensis* exhibit the 'typical' physiological responses (cortisol, glucose, lactic acid, osmolality, and ions) induced by vibration stress. Our data suggested that chronic vibration stress causes substantial stress in the animal, and in the particular, the persisting elevated levels of AST and ALT observed would be expected to adversely affect. The high hematological characteristics during all experimental period showed that the *P. sinensis* couldn't adapt to chronic stimuli provoked by vibration stress.

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