J. Appl. Anim. Res. 37 (2010) : 241-245

Effects of Chronic Vibration Stress on Liver, Kidney and Testes of the Soft-Shelled Turtle *Pelodiscus sinensis*

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(Received July 31, 2009; accepted April 24, 2010)

Abstract

Hur, J.W. and Lee, J.Y. 2010. Effects of chronic vibration stress on liver, kidney and testes of the soft-shelled turtle *Pelodiscus sinensis*. J. Appl. Anim. Res., 37: 241-245.

The effects of chronic vibration stress 61.6±16.6 dB V for 30 min at 2h intervals, 08.00–18.00h on the histology and physiology of liver, kidney and testes of the soft-shelled turtle ***Pelodiscus sinensis*** *was examined.* ***P. sinensis*** *exhibited typical physiological responses (increased cortisol and glucose levels) to vibration stress. In the SG, hepatocytes, kidney and testes showed abnormal tissue morphology compared to the NSG. In the SG, gonadosomatic and hepatosomatic indices (GSI and HSI) showed significant differences relative to before the start of the experiment and to the NSG. These results suggest that chronic vibration caused substantial stress in the animals and* ***P. sinensis*** *could not adapt to chronic stimuli provoked by vibration stress.*

Key words: Soft-shelled turtle, *Pelodiscus sinensis*, vibration stress.

Introduction

The soft-shelled turtles, *Pelodiscus sinensis,* are frequently used for medicinal purposes or consumed as gourmet and health food in Korea, Japan and China. In a natural setting, *P. sinensis* is a secretive animal that is very sensitive to noise and vibration. *P. sinensis* farms should, therefore, be located where the turtles are unlikely to be affected by environmental stresses, although some exposure to noise and vibration is inevitable.

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Investigations of the responses to stress have mainly been conducted on hormone secretion due to handling and capture (Gregory *et al*., 1996; Cash *et al*., 1997; Mahmoud and Licht, 1997; Gregory and 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 are available on the histological responses of this species to acute and chronic stress.

In the present study changes in histological structure and hematological characteristics occurring in *P. sinensis* during vibration stress, have been reported.

J. Appl. Anim. Res. 0971-2119/2010/$10.00

Materials and Methods

Soft shelled turtles (*P. sinensis,* 3-4 year, 16.8±1.8 cm carapace length, 0.7±0.2 kg) were purchased from a farm and habituated in a lab aquarium tank for 2 weeks before use in the study. *P. sinensis* in stress group (SG, *n*=20) were placed in a tank (2 ton, FRP, raceway style) and an electric vibrator was attached to one end of tank. The vibration stress was turned on for 30 min at 2 h intervals during day time (08:00-18:00) for 28 d, at an average vibration level of 61.6±16.6 dB V and an average noise level of 73.6±4.8 dB A during day time, and at an average vibration level of 25.0±5.8 dB V and an average noise level of 58.7±7.2 dB A in normal condition.

In order to exclude other stresses than vibration, the lab was constantly kept dark, with no other disturbances, and the water temperature was automatically regulated at 25C. The *P. sinensis* in the NSG (*n*=20) were maintained under the same environmental conditions excluding vibration for 28 d.

The liver, kidney and testes were removed, tissue samples were fixed in 10% neutral formalin solution for 24 h and refixed in Bouin's solution for 24 h. Samples were prepared in 6μm thick paraffin sections, placed on slides, stained with hematoxylin and eosin Y-phroxine B.

Blood was sampled during 1 min without anesthesia from the neck blood vessel complex using heparinized syringes at d 0 and at 7, 14, 21 and 28 d after vibration stress, plasma was separated and stored in a deep freeze (–70C) until analysis. Plasma cortisol concentrations were determined in 50μl samples using radioimmunoassay kits (Coat-A-Count TKCO Cortisol RIA Kit; DPC, USA) according to the Davis *et al.* (2002) method. Mixtures of samples in 100 ml of antiserum were incubated for 45 min at 37C and then 1000 ml of separation reagent was added. The mixtures were placed in a refrigerator at 4C 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). The plasma glucose concentration was analyzed, according to Park *et al.,* 2009; (Kit 510, Sigma, St Louis, MO, USA), through, evaluating the production of H_2O_2 by glucose oxidase in the presence of o-dianisidine as an absorbance increase at 450 nm. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed using an automatic chemistry analyzer (Hitachi 7180, Hitachi, Japan).

The gonadosomatic and hepatosomatic indices, GSI (total gonad weight/total body weight×100) and HSI (total liver weight/total body weight×100), respectively, were calculated.

Data were analyzed by a one-way ANOVA in the SPSS statistical package. Means were separated by using Duncan's multiple range test.

Results and Discussion

Plasma cortisol and glucose concentration in the stressed group increased ($P<0.05$) during the experimental period (Fig. 1). Long-term studies on chronic physiological responses to vibration are rare. Some short-term studies have reported increased cortisol levels related to stress in turtles. This increase was reported to be due to capture in sea turtles, (Jessop *et al.*, 2002; 2004). However, we believe that long-term exposure to vibration affects the animals more seriously than capture, as cortisol levels remained high for 28 d after the cessation of stress.

Figure 2A shows hepatocytes of turtles in the NSG, arranged in a radial shape of hepatic cords around central veins in the center, which are separated by sinusoidal capillaries creating phases of structure. The hepatocytes around the portal vein have clear borders, with a high density of cells. The hepatocytes of turtles in the SG show radial hepatic cords around a central vein; however, the form is less complete than that of hepatocytes in the NSG (Fig. 2B–C). As hepatocytic degeneration occurs, hepatic

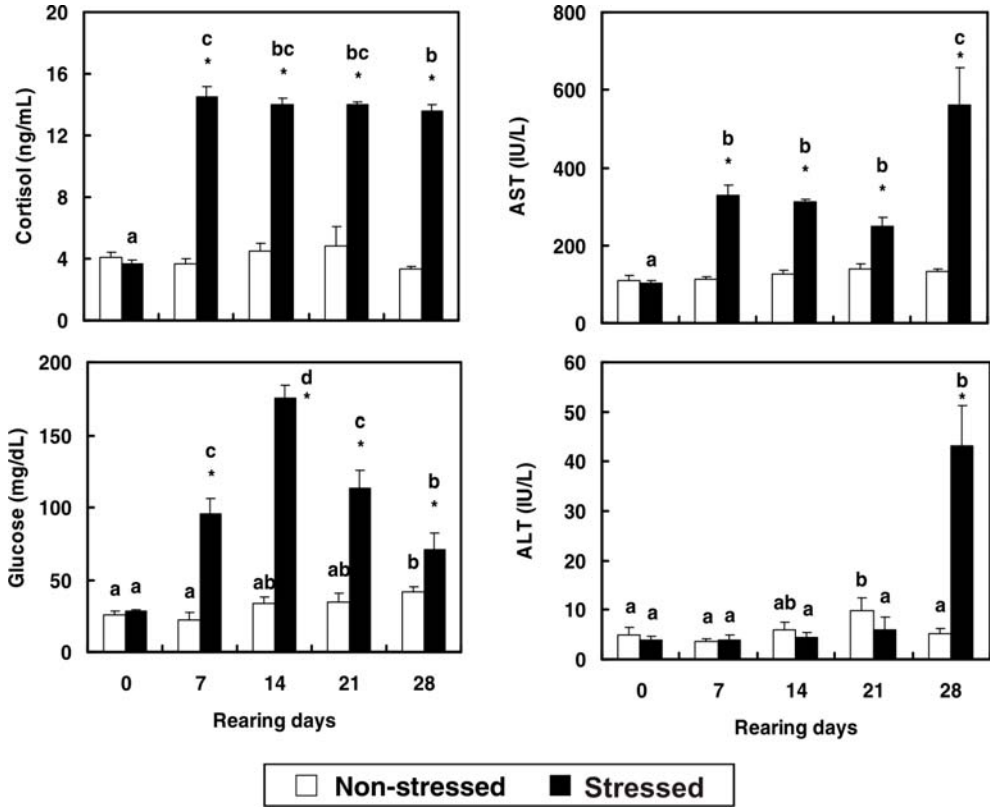

Fig. 1. Levels of the plasma cortisol, glucose, AST and ALT of non-stressed and stressed groups in soft-shelled turtle *P. sinensis*. Shared alphabetic letters on shaded bars indicate lack of significant difference *indicates significant differences between groups at equivalent days ($P<0.05$).

cords begin to appear disintegrated and vacuolation around the central vein becomes more obvious. Centrillobular congestion was also observed in some regions. Intercellular junctions were indefinite around the portal vein, with an increase in accumulation of melanomacrophages (MM).

MMs appear as small to large round or oval structures, which are easily distinguished from the surrounding lymphatic tissue, whereas under an electron microscope, they appear as groups of macrophages (Agius and Roberts, 2003: Hur *et al*., 2006). In fish, MMs are observed in normal individuals but are more numerous in physiologically abnormal states due to disease or stress; moreover, in these states, the number, size and shape of MMs vary (Agius and Roberts, 2003).

In our study, AST and ALT levels in the SG were significantly elevated by chronic vibration stress (Fig. 1), suggesting that vibration places physiological burdens on the liver and spleen.

In the kidney tissue, normal renal corpuscles were found in the NSG (Fig. 3A). In addition, Bowman's space, the visceral layer and podocytes were complete around the glomerulus. In contrast, the glomerulus and Bowman's capsule of turtles in the SG showed an indistinct shape and the epithelium of podocytes surrounding the inner layers of Bowman's capsule was damaged. The inner and outer layers of the glomerulus were not distinguished. Bowman's space and the inner wall were closely attached, which made it difficult to observe each individually. The glomerulus, however, was stained with eosin (Fig. 3B). The shape of the renal tubule showed a low density due to destruction, multiple tissue damages and vacuolation. The renal tubules showed an irregular shape and pattern,

unlike in the NSG. Capillaries and cells in the renal tubules were exposed and open as a result of vacuolation. A higher MM deposition was observed in the SG than in the NSG.

During histological observations of the testes in both the SG and NSG, wavy patterns were found in the NSG with fully developed sperm (Fig. 4A). In the SG, mature sperm were found, most of which remained at the spermatoblast stage. Vacuolation was also evident in the testes (Fig. 4B).

The GSI in the SG demonstrated a declining pattern as the experiment progressed, and the values were lower than in the NSG (Table 1). At the start of the experiment, eggs of females were immature and of variable sizes. We found that the numbers of eggs in larger individuals were

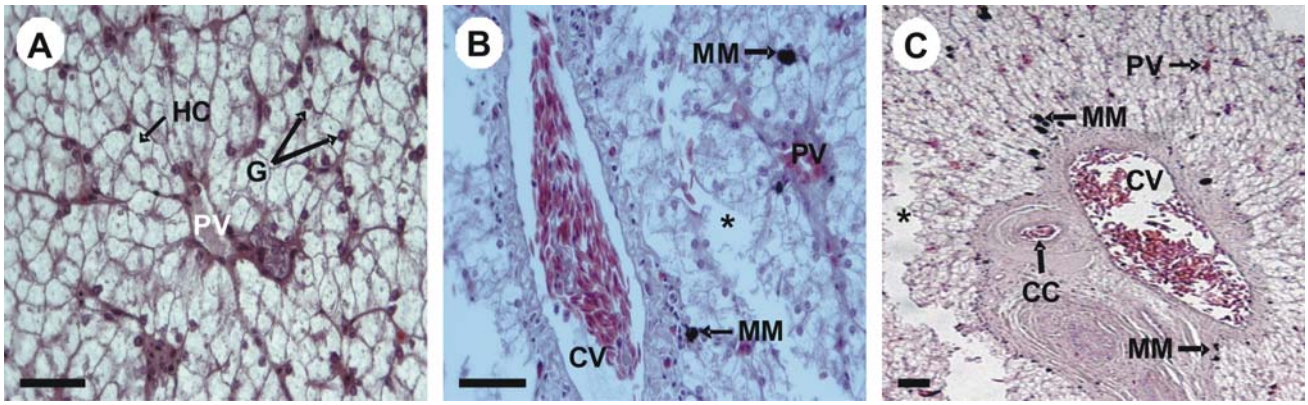

Fig. 2. Histological observations on the hepatocytes of non-stressed and stressed groups in soft-shelled turtle *P. sinensis*. A: NSG (0 d), B, C: SG (28 d). CC: centrillobular congestion, CV: central vein, G: glycogen, HC: hepatocyte, MM: melano-macrophage, PV: portal vien. *Vacuolation. Bars: 50 μm.

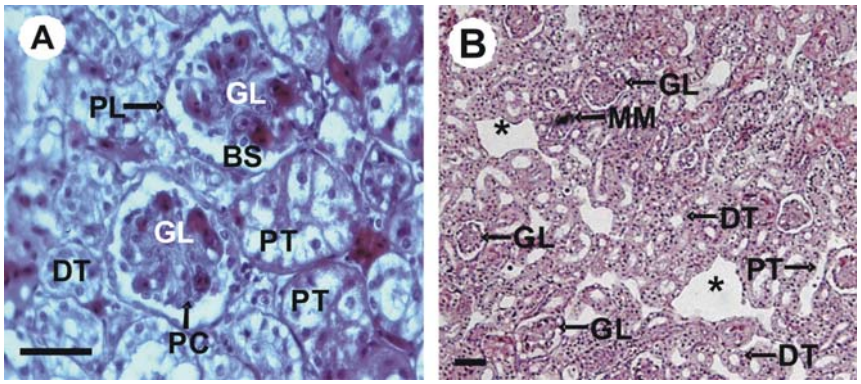

Fig. 3.

Histological observations on the kidney of non-stressed and stressed groups in soft-shelled turtle *P. sinensis*. A: NSG (0 d), B: SG (28 d). BS: Bowman's space, GL: glomerulus, DT: distal tubule, MM: melano-macrophage, PC: podocyte, PL: parietal layer, PT: proximal tubule. *Vacuolation. Bar: 50μm.

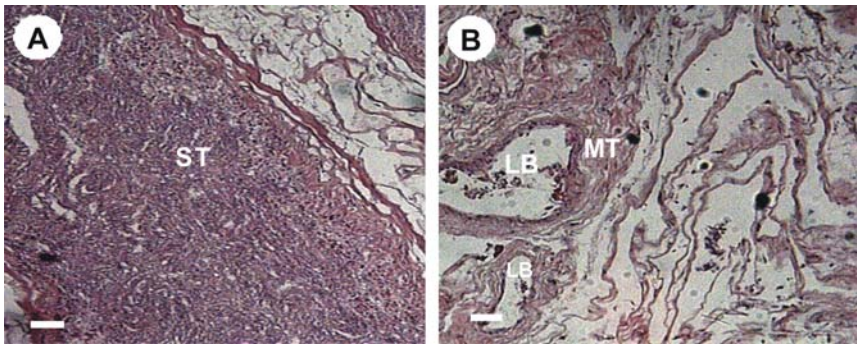

Fig. 4.

Histological observations on the testis of non-stressed and stressed groups in soft-shelled turtle *P. sinensis*. A: NSG (0 d), B: SG (28 d). LB: lobuli testis, MT: mediastinum testis, ST: spermatids. Bar: 50 μm.

Table 1
Variations of gonadsomatic index (GSI) and hepatosomatic index (HIS) of gonad and liver in soft-shelled turtle *P. sinensis*

Rearing days	GSI				HIS			
	Female		Male		Female		Male	
	Non-stress	Stressed	Non-stress	Stressed	Non-stress	Stressed	Non-stress	Stressed
0	2.87±0.24	2.87±0.24	1.14±0.05	1.14±0.05	1.93±0.21	1.93±0.21	2.16±0.26	2.16±0.26
28	2.70±0.19	1.82±0.89*	1.30±0.06	0.90±0.08*	1.76±0.47	2.65±0.52*	2.47±0.54	3.49±0.63*

*Indicates significant differences between groups at equivalent days ($P<0.05$).

dramatically decreased on the last day of the experiment (d 28). The value of the HSI in the SG during the final stages of the experiment showed a significant increase compared to the NSG.

It is concluded that chronic vibration caused substantial stress to the animals. The abnormal tissue morphology showed that *P. sinensis* was unable to adapt to chronic stimuli provoked by vibration stress.

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जे.डब्ल्यू. हूर, जे.वाई. ली। कोमल कवच कछुए (पेलोडिस्कस साइनेन्सिस) के यकृत, वृक्क और वृषणों पर दीर्घकालिक कंपन प्रतिबल का प्रभाव।

कोमल कवच कछुए (पेलोडिस्कस साइनेन्सिस) के यकृत, वृक्क और वृषणों के उत्तकी और दैहक्रियकी पर प्रतिदिन 08.00 बजे से 18.00 बजे तक 2 घंटे के अंतर पर 30 मिनट तक 61.6±6.6 डीबीवी दीर्घ कालिक कंपन प्रतिबल के प्रभाव की जांच की गयी। कछुए में कंपन प्रतिबल का प्ररूपी दैहक्रियकी प्रभाव (कोर्टिसाल और ग्लूकोज के स्तरों में वृद्धि) था। अप्रतिबल वर्ग (एनसीजी) की अपेक्षा प्रतिबल (एसजी) प्रभावित वर्ग की यकृत कोशिकाओं वृक्क एवं वृषणों में असामान्य उत्तक संरचना पायी गयी। एसजी के हिपैटोसोमेटी इंडेक्स (एचआई) और गोनेडोसोमेटी इन्डेक्स (जीआई) में एनसीजी प्रयोग प्रारंभ होने की तुलना में सार्थक अंतर पाया गया। परिणामों से ज्ञात होता है कि दीर्घकालिक कंपन का बहुत प्रतिबल प्रभाव पड़ा और कछुओं का अनुकूलन नहीं हो सका।