The Histological Structure of the Liver and Pancreas and Distribution of Glucagon of Pancreas in the Siberian Tiger (Panthera tigris altaica)

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Abstract: Glucagon is the hyperglycemic factor that opposes the action of insulin. It stimulates the breakdown of liver glycogen and increased glucogenic gene. Glucagon is located in the tissues of the pancreases of many animals. However, the distribution of glucagon has not been investigated in the pancreases of the tiger. The present study was performed to describe the histological structure of the liver and pancreas and the location of glucagon of pancreas in the 8 days old Siberian tiger. The tissues of the liver and pancreas were collected from three 8 days old Siberian tigers that died of starvation. The weight was recorded and the histological sections were made and stained with HE. The localization of glucagon in tissue was detected by immunohistochemical two-step PV-9000. The results show that the average weight of the liver and pancreas was approximately 39.06 and 1.97 g, respectively. The tissue structure of the liver consisted of hepatic lobulation and portal area. Central vein, hepatocyte tube and hepatic sinusoid and lymphatic tissue were found in hepatic lobulation. Interlobular veins, interlobular artery and interlobular bile duct were presented in portal area. The pancreas was basically made up of exocrine portion and pancreas islet. The exocrine secretion of the pancreas included many pancreatic aciniuses and centroacinar cell was observed in the pancreatic acinus. Glucagon-immunopositive cells were mainly located in the pancreas islet. The current study preliminarily revealed the histological features of the liver and pancreas and the distribution of glucagon in the pancreas of 8 days old Siberian tiger which requires further investigation.

Key words: Siberian tiger, liver, pancreas, histological structure, glucagon

INTRODUCTION

Siberian tiger (Panthera tigris altaica) which is also known as the Amur, Korean, Altaic, North Chinese or Ussuri tiger is a subspecies of tigers. The dual threats of illegal poaching and the destruction of its habitat have brought this species to the brink of extinction with a population of 450 in the world and 20 in China (Hu et al., 2006).

A few studies have reported on tiger oocoyte, embryo, genetics and cloning (Gjorret et al., 2002; Hu et al., 2006; Putranto et al., 2007; Liu et al., 2010). However, no studies have identified the histological structure of the liver and pancreas and cytological characteristics of glucagon of pancreas in the Siberian tiger. With the restricted population of the tiger, it would be a challenge for scientists to learn and familiarize themselves with the morphologic characteristics of glucagon in the tiger.

Glucagon is a 29 amino acid peptide. Glucagon restores normal blood glucose levels by increasing hepatic glucose production and disruption of glucagon signalling results in hypoglycemia (Qureshi et al., 2004; Sloop et al., 2004; Lou et al., 2011). Glucagon secretion is suppressed in response to elevated blood glucose levels whereas reduced blood glucose enhances glucagon release. Glucagon is the principal hormone stimulating hepatic glucose production. Previous studies have analyzed the pancreatic development (Manakova and Titbach, 2007; Chandavar and Naik, 2008; Jeon et al., 2009; Carlsson et al., 2010) and endocrine cells (Yukawa et al., 1999; Ku et al., 2004; Ku and Lee, 2006). In addition, it has also been reported that glucagon is distributed in the pancreatic islet of many animals with a regionally specific pattern in the pancreas (Gustavsen et al., 2008; Huang et al.,...
However, the regional distribution of gluca
gon in the pancreas of Siberian tiger remains unclear.

The present study was the first observation of the
histological structure of the liver and pancreas and
localization of gluca
gon in the pancreas in the neonatal male Siberian tiger. The results that are presented here will
help us understand the biological characteristics of this
endangered species.

MATERIALS AND METHODS

Animals and sample collection: The samples were
collected from three male Siberian tigers that died
accidentally at the Hefei Wildlife Park in Anhui province
in China. No abnormalities were found. The tigers
were 8 days of age. After death, the abdominal cavity was
opened and the liver and pancreas were dissected and
immediately brought to the laboratory in saline at 4°C.
Subsequently, the weight of the liver and pancreas were
recorded.

Preparation of sections: A small tissue sample of the liver
and pancreas was collected and fixed for 24 h in 4%
paraformaldehyde (Klimpath, Geel, Belgium) at 4°C and
after paraffin embedding, 5 μm serial sections were
prepared, placed on poly-L-lysine-coated glass slides and
dried for 1 h at 60°C for histological and
immunohistochemical analyses.

Histological evaluation: The sections of each tissue
were stained with Hematoxylin and Eosin (HE) for light
microscopic examination of the normal histological
structure.

Immunohistochemical procedure: Each section was
deparaffinized, rehydrated and immunostained by using
standard procedures (PV-9000 Method) with rabbit
anti-gluca
gon (1:100 dilution, code BA1619, Zhongshan
Goldenbridge Biotechnology Co., Ltd. Beijing, China)
as the primary antibody. The secondary antibody was
biotinylated goat anti-rabbit IgG (1:100 dilution, Golden
Bridge International, Inc., USA). Samples were
developed using standard DAB reagents (Zhongshan
Goldenbridge Biotechnology Co., Ltd. Beijing, China).
After immunostaining, the sections were observed under
a light microscope. The negative controls were produced
by substituting the primary antibody with 0.01 M PBS.

RESULTS

The structure of the liver: The average weight of liver
was about 39.07 g. The histological structure of the

liver consisted of hepatic lobulation and portal area
(Fig. 1a). The lobulation was not well defined because
the interlobular connective tissue was not developed
(Fig. 1b). The central vein presented in the centre of
hepatic lobulation, the hepatic plate was radially arranged
around the central vein, the reticular vascular between the
plate was hepatic sinusoid, the wall of sinusoid formed by
endothelial cell. Kupffer cells were found in the sinusoids
(Fig. 1d). The hepatic plate was arranged with the single
hepatocytes (Fig. 1d). The primary cell in the liver was the
hepatocyte. There was polarity of hepatocytes. The portal
area consisted of interlobular veins, interlobular artery
and interlobular bile duct (Fig. 1c). Lymphatic tissue was
found in the lobulation (Fig. 1d).

The structure of the pancreas: The average mass of the
pancreas was 1.79 g. The pancreas was divided into many
pancreatic lobules by surface connective tissue (Fig. 2a).
The pancreas consisted of exocrine portion and endocrine
portion. The exocrine secretion of the pancreas was cells
which the nuclei was big and round, there are centroacinar
cell was found in the narrow acinar lumen (Fig. 2b). The
catheter was responsible for carrying pancreatic juice to
duodenum. The walls of all catheters were surrounded by
a single layer of epithelial cells. The islets were the
docrine portion of the pancreas which the specific cell
types could not identity by HE (Fig. 2c and d).

Location of gluca
gon in the pancreas: In the pancreas,
 gluca
gon-immunoreactive cells were and mainly localized
were detected in the pancreatic duct portions. However, glucagon staining was not observed when the glucagon antibody was replaced with PBS (Fig. 3b).

**DISCUSSION**

For the first time, the current study showed the structural details in the liver and pancreas and the distribution of glucagon in the pancreas of the 8 days old Siberian tiger.

The hepatic lobulation and portal area were presented in the liver and the lobulation was not well defined because of thin interlobular connective tissue in the Siberian tigers which is the same to the structure of the liver in the cattle, sheep, horse and birds (Peng, 2009).

But interlobular connective tissue in the liver was developed in the pig, cat and camel (Shen, 2002). Abundant lymphocytes were distributed in the hepatic lobulation which is different from other mammals, indicating local immune function is very strong in the liver of the Siberian tigers. It is generally known that the pancreas of vertebrates is subdivided into exocrine and endocrine portions. The pancreas of the Siberian tiger had the same features.

Glucagon is synthesized in the A cells of the pancreas and regulates blood glucose levels (Ku et al., 2002a, b). In the present study, glucagon-immunoreactive cells were found in the central regions of pancreatic islets. Although, glucagon-immunoreactive cells have been found in the central regions of equine pancreatic islets (Ku et al., 2002a) including the present study, species-dependent variations have been reported. Glucagon-immunoreactive cells were found in the mantle and peripheral regions of mammalian pancreatic islets, exocrine portions and pancreatic duct (Krause et al., 1989; Sasaki et al., 1991; da Mota et al., 1992; Edwin et al., 1992; Gomez Dummm et al., 1996; Wieczorek et al., 1998). In addition, it was also reported that under specific disease conditions such as obese (diabetic condition) mouse, glucagon-immunoreactive cells were intermingled with insulin-immunoreactive cells in the central regions of pancreatic islets in contrast, normal non-obese littermates showed a peripheral localization of these immunoreactive cells (Ku et al., 2002b). In the red-bellied frog (*Bombina orientalis*), glucagon-immunoreactive cells were also detected as single cells or as clusters but in the case of clusters, two distributional patterns were detected—a central core type and a marginally distributed type (Lee et al., 2003). These findings are considered to represent a species-dependent unique distributional pattern.
CONCLUSION

Researchers reported for the first time the histological structures of liver and pancreas and the distribution of glucagon in the pancreas of male Siberian tigers aged at 8 days old. However, these results were based on histology. Further research is required to understand the physiology at the cellular and molecular level.

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REFERENCES