Acta Med. Okayama, 2003
Vol. 57, No. 2, pp. 53–58

Acta Medica Okayama
http://www.lib.okayama-u.ac.jp/www/acta/

Original Article

Increase of S-100 Protein-positive Stellate Cells in the Anterior Pituitary of Chronic Alcoholic Patients with Fatty Liver or Fatty Cirrhosis

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Healthy subjects 40 years old were used as controls in a study of stellate cells (S-100 protein-containing cells, or S-100 cells) in subjects with chronic alcoholism and fatty liver or fatty cirrhosis. S-100 cells were sparsely found in the adenohypophysis of control subjects, and these cells sometimes formed small clusters. However, in chronic alcoholics with fatty liver or fatty cirrhosis, the number of stellate cells in the anterior pituitary tended to be 17 times higher than it was in the control group. No increase in the number of S-100 positive cells that constitute the large and small follicles in the intermediate pituitary. The physiological function of the S-100 protein has not yet been identified. The fact that an increase in prolactin-secreting and growth hormone-secreting cells, as well as a decrease in gonadotrophs were observed in the hypophysis of alcoholics suggests that the function of stellate cells may be closely related to these phenomena. Our results also imply that the stellate cells found in the anterior and intermediate pituitary differ in function although they both produce S-100 proteins.

Key words: S-100 protein, pituitary, alcoholism

S-100 protein is generally regarded to be a protein specific to glial cells [1, 2], especially cerebral astroglial cells [3]. Moore [1] as well as Moore and McGregor [4] successfully isolated the specific S-100 protein from the bovine cerebral cortex. They also demonstrated biochemically that glial cells contain a large amount of S-100 proteins. This specific cerebral protein is present in the stellate cells (agranular cells) and in the folliculo-stellate cells of the adenohypophysis and in the follicle-forming cells of pars intermedia [5]. In rats, the protein is also found in the peripheral cells of the adenohypophysis (*i.e.*, the cells lining the residual Rathke's pouch) [6, 7]. Several hypotheses have been proposed regarding the function of S-100 cells; such as role resembling to adrenocorticotropic hormone (ACTH)-secreting cells [8–11], and their roles as stem cells [12], undifferentiated granular cells [13], supporting cells [14], phagocytic cells [15, 16], and nursing cells [17] of the adenohypophysis. Ishikawa *et al.* [18] have reported that S-100 protein stimulates the prolactin-producing clone to secrete prolactin *in vitro*. However, to date, there has been no report regarding either the function of S-100 protein-containing cells in the human hypophysis or the physiological role of S-100 protein *in vivo*.

In this study, using immunohistochemical methods,

Received September 9, 2002; accepted November 5, 2002.
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we found that there was an increase in the number of stellate cells (S-100 protein-containing cells) in the anterior pituitary of chronic alcoholics with fatty liver or fatty cirrhosis. Here, we report these results together with our hypothesis regarding S-100 cell function.

Materials and Methods

Specimens. Specimens of the hypophysis from 9 female and 27 male patients in their forties with alcoholic fatty liver or fatty cirrhosis, together with 12 controls of the same age group (obtained from autopsy cases of cerebral hemorrhage, coronary artery sclerosis, or traffic accident) were examined in this study. Although a diagnosis of alcoholic fatty liver and fatty cirrhosis is difficult to establish, we included only cases of advanced fatty liver and fatty cirrhosis based on the following criteria reported by Takahashi *et al.* [19]: 1) The patient had alcohol dependency; 2) Serological tests for HA (hepatitis A) antibody, HBs (hepatitis B surface) antigen, HBc (hepatitis B core) antibody, and HCV (hepatitis C virus) antibody were all negative; 3) Histological studies showed large lipid droplets in hepatocytes and diffuse fatty liver; 4) The liver was grossly and histologically classified as type F according to Miyake's classification (almost even-sized regenerating nodules, and narrower fibrous septum than that associated with viral cirrhosis); 5) Ascites and congestive splenomegaly were present.

The hypophyses obtained at autopsy were fixed for two to three days in 10 % phosphate buffered formalin solution at 4 °C. Afterwards, they were dehydrated with ethanol and embedded in paraffin. Four-μm frontal serial sections were cut from the anterior, middle, and posterior portions of the hypophysis.

Immunostaining procedure for light microscopy. After deparaffinizing, the sections were immersed in 5 % normal goat serum diluted in 0.1 M phosphate buffered saline (PBS) for 10 to 15 min at room temperature in order to remove any non-specific antibody binding. The sections were washed in 0.1 M PBS several times. Afterwards, rabbit anti-bovine S-100 protein antibody (Polyclonal, whole (both α and β subunits), 1: 5,000 dilution; supplied by the Department of Anatomy, Jikei University, School of Medicine) [20] was applied to the sections and the slides were incubated in a humid atmosphere at 30 °C for 6 to 9 h. After several washes with PBS, immunostaining was performed by the ABC method using goat anti-rabbit Ig (Dako, Tokyo, Japan) as the second antibody and DAB as the coloring agent. The nuclei were counterstained with hematoxylin.

Semi-quantitative measurement of S-100 protein-containing cells. Frontal serial sections were cut from the anterior, middle, and posterior portions of the hypophysis and the sections were immunostained for S-100 cells (both cytoplasm and nuclei were stained). The area of positively stained cells was measured 3 times using an Olympus Color Image Analyzer (VIP-21CH, Olympus, Tokyo, Japan). The final result is expressed as the proportion of the area of S-100 cells to the whole area of the anterior pituitary.

Results

In the pituitary of the control group, a small number of stellate cells (S-100 cells) were scattered mainly in the outer portion of the anterior pituitary (Fig. 1). These cells were polygonal or stellate in shape and sometimes clustered in the anterior pituitary to form a colony (Fig. 1). Under close observation, the stellate cells were often found in the central part of the lobules of the hypophysis and extended cytoplasmic processes to the basal membrane of the lobule (Fig. 1).

In the hypophysis of alcoholics with fatty liver, the stellate cells were enlarged; the number of these cells was increased and the cytoplasmic processes were more elongated than those in the control group (Fig. 2A). Some of the stellate cells were observed as forming long processes around other glandular cells, almost as if to engulf these cells (Fig. 2B). The stellate cells formed clusters. As in the control group, the stellate cells tended to gather in the center of lobules and send cytoplasmic processes toward the basal membrane of other glandular cells (Fig. 2A).

There was a marked increase in the number of stellate cells in the pituitary of both male and female patients with alcohol-derived fatty cirrhosis as compared to those with fatty liver due to excessive alcohol consumption (Fig. 3). In the patients with fatty cirrhosis, some of the stellate cells in the anterior pituitary tended to engulf the surrounding glandular cells, although the stellate cells formed follicles similar to those of the S-100 cells in the pars intermedia (Fig. 4). Other stellate cells gathered in the center of the lobules and extended long cytoplasmic processes between the glandular cells and toward the basal membrane of the lobules (Fig. 3).

The pars intermedia of the human hypophysis contains follicles (cysts) of various sizes. The follicular epithelium

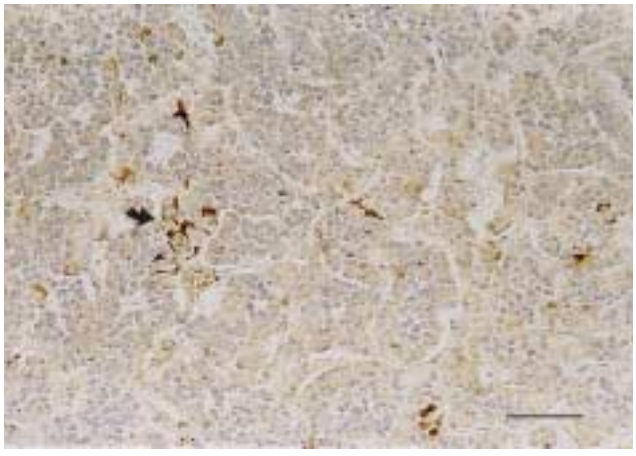

Fig. 1

Fig. 1 Micrograph showing immunostaining of S-100 cells in the peripheral area of the adenohypophysis in a control sample. The arrowhead points to a long cellular process of the S-100 cell extending towards the basal membrane of the lobule. Bars indicate 100 μm.

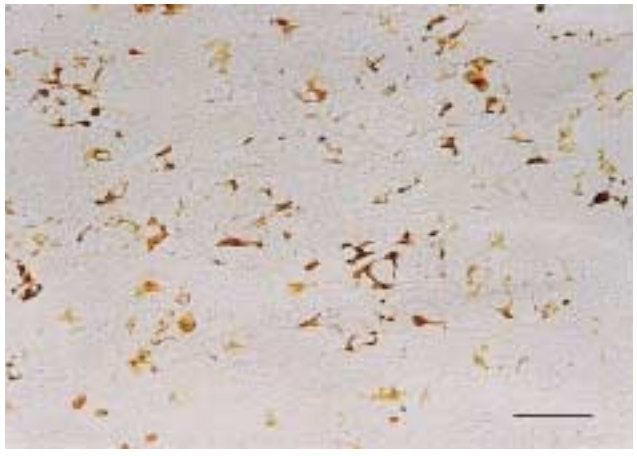

Fig. 2A

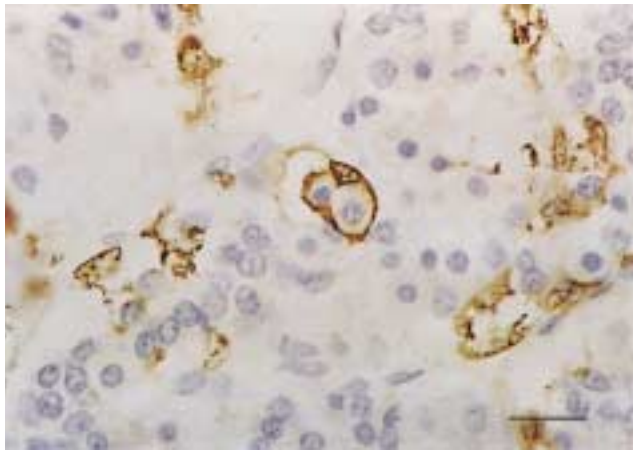

Fig. 2B

Fig. 2 (**A**) Micrograph showing an increase in S-100 cells in the adenohypophysis of a patient with fatty liver. Some S-100 cells are located in the center of the lobule and extend long processes toward the basal membrane. Bars indicate 100 μm. (**B**) Micrograph showing a typical picture of S-100 cells engulfing other cells. Bars indicate 30 μm.

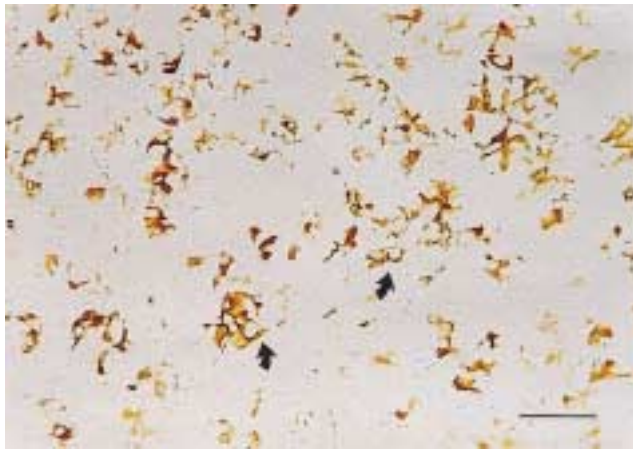

Fig. 3

Fig. 3 Micrograph showing S-100 cells in the pituitary of a patient with fatty cirrhosis. Numerous S-100 cells occupy the adenohypophysis and engulf other glandular cells. The arrowhead shows a typical engulfed cell. Bars indicate 100 μm.

was usually composed of S-100 cells (Fig. 5A) with the cells at the inner surface in contact with the follicular (cystic) fluid (Fig. 5B). The number of S-100 cells in the pars intermedia of patients with alcoholic fatty liver or cirrhosis showed no significant increase over that of the control group.

The proportion of the area of stellate cells to the whole anterior pituitary area was 2.10 ± 0.39 % in the female controls and 1.31 ± 0.42 % in the male controls. In the group of patients with alcoholic fatty liver, the proportion was 9.84 ± 3.72% in females and 11.2 ± 3.63% in males. In patients with fatty cirrhosis, the proportion was 20.1 ± 4.60% in females, and 20.6 ± 4.38% in males (Fig. 6).

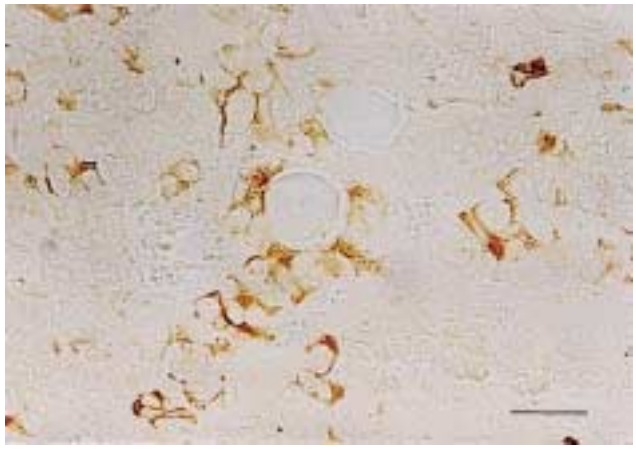

Fig. 4

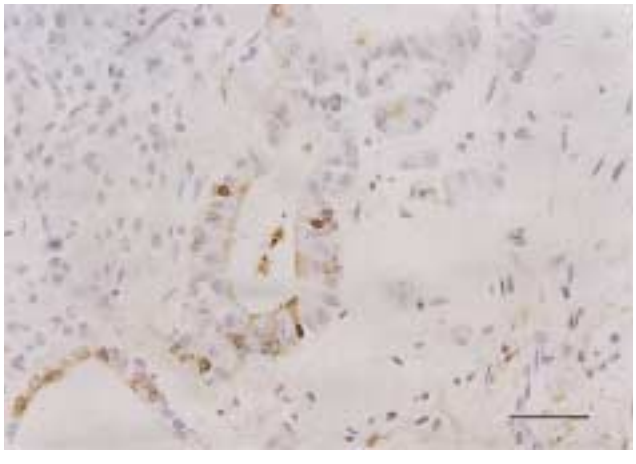

Fig. 5A

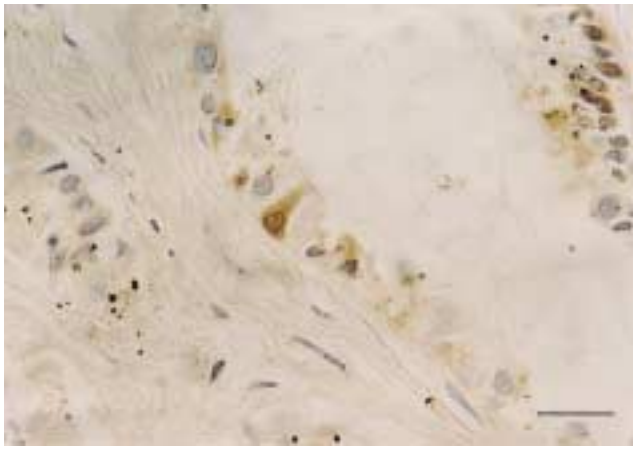

Fig. 5B

These findings suggest that the area of the entire anterior pituitary occupied by stellate cells had increased tenfold in the patients with alcoholic fatty liver and was seventeen times larger than that of the control group in the patients with fatty cirrhosis due to excessive alcohol consumption.

Discussion

In this study, anti-bovine S-100 protein antibody was used instead of anti-human S-100 protein antibody. Both antibodies immunostained the same cells in the anterior pituitary; however, the anti-bovine S-100 antibody stain was more intense than that of the anti-human S-100 antibody. The S-100 protein-containing cells in the human hypophysis were polygonal or stellate in shape. Follicle-

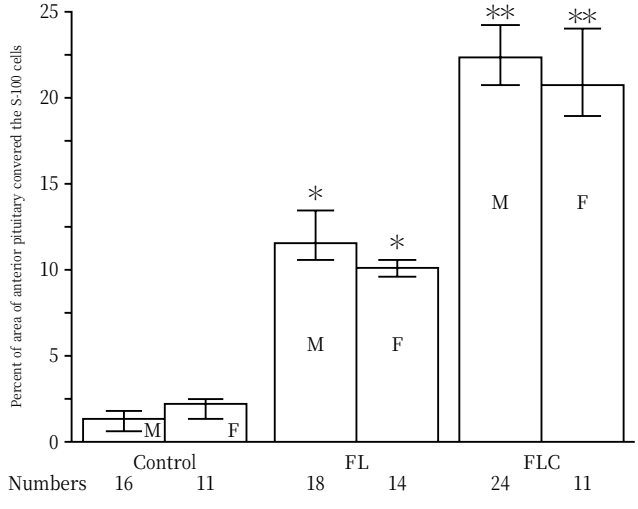

Fig. 6 Graph showing the median ± SEM of the area occupied by S-100 cells in the adenohypophysis (obtained from subjects in their forties). FL, fatty liver *; FLC, fatty cirrhosis **; M, male; F, female; *, $P < 0.05$; **, $P < 0.02$.

Fig. 4 Micrograph showing S-100 cells in the adenohypophysis of a patient with fatty cirrhosis. The S-100 cells, while forming follicles similar to those of S-100 cells in the pars intermedia, also show engulfment of other glandular cells. Bars indicate 30 μm.

Fig. 5 (**A**) The follicular epithelium in the pars intermedia consists of immunostained S-100 cells (**A**). (**B**), represents a higher magnification of (**A**). The S-100 cells engulf other granular cells and their processes cover the inner surface of the follicular epithelium. Bars indicate 100 μm in (**A**) and 30 μm in (**B**).

forming S-100 cells are commonly found in the rat hypophysis, but they are rarely observed in human adenohypophysis. Since anti-S-100 antibody immunostains both the nucleus and the cytoplasm, the use of an image analyzer is the best method to quantitatively measure the S-100 protein-positive cells that may assume polygonal, stellate, or follicular form.

In humans, S-100 cells are known to appear in the pars intermedia of the fetal hypophysis at 22 weeks of gestation, whereas these cells first appear in the adenohypophysis around 7 years of age, and their number increases with age [21]. We examined the pituitary of subjects in their forties because the stellate cells (S-100 positive cells) are still present in small numbers in the normal adenohypophysis of this age group. Our results showed that there was an increase in the number of stellate cells in the anterior pituitary of patients in their forties with either alcoholic fatty liver or cirrhosis.

In alcoholics with fatty liver or cirrhosis, the number of acidophils growth hormone- and prolactin-secreting cells is known to increase [22, 23]. The cause of this increase is not yet known. However, gynecomastia, commonly seen in cirrhotic patients, is caused due to the liver's reduced ability to break down estrogen, which leads to high levels of estrogen and androgen in the blood; this may result in an increase of prolactin-secreting cells [22]. Furthermore, in the liver, which is a target organ of growth hormones, insulin-like growth factor-I, produced in response to stimulation by growth hormones, is reduced in the case of either fatty liver or fatty cirrhosis; this reduction may act as negative feedback, there by causing an increase in the number of growth hormone-secreting cells. On the other hand, the number of gonadotrophs is known to be decreased in the hypophysis of alcoholics [24]. Ishikawa *et al.* [18] has established a novel clone of S-100 protein-containing cells from the rat adenohypophysis. This clone synthesizes and secretes S-100 proteins and regulates the secretion of prolactin *in vitro*. It is already known that the number of prolactin-secreting cells is increased in chronic alcoholics with fatty liver or cirrhosis, and a report by Ishikawa *et al.* [18] supports our current study that the number of S-100 cells is also increased in chronic alcoholics as compared to controls. However, we observed no increase in the number of S-100 cells in the pars intermedia. This result suggests that the S-100 cells of the adenohypophysis and pars intermedia differ as regards function. Just as dopamine [25] or prolactin inhibiting factor [26] exerts negative feedback on prolactin-secreting cells of the adenohypophysis, S-100 cells may also be receiving negative feedback from the hypothalamus, with an increase in S-100 cells accompanying the atrophy of the hypothalamus that has been observed in chronic alcoholics [27].

Immunohistochemical studies using serial sections have shown that human S-100 cells are entirely different from the hormone-producing cells of the human adenohypophysis (data not shown). However, an immunohistochemical study of the goat adenohypophysis has revealed the presence of growth hormone in S-100 cells [20]. This result is interpreted as suggesting the possibility that S-100 cells may belong to a class of stem cells or progenitor cells of growth hormone-producing cells [20].

The S-100 protein is composed of 2 biochemically identifiable entities [28, 29, 30]. Both components show the same serum immunological reaction as that of the whole S-100 protein [31]. The tissue immunostaining ability of the anti-serum used in the current study (diluted with PBS) was completely abolished after incubation with over 4 μg of purified S-100 protein [21], indicating that the anti-serum used here specifically reacted with S-100 protein.

Past studies have shown that a wide distribution of substances are immunoreactive with anti-bovine S-100 protein antibody in rat folliculo-stellate cells [3, 6]. The folliculo-stellate cells have been considered to possess the following characteristics: 1) supportive function [14, 32, 33]; 2) phagocytic scavenger function [34]; 3) ion transport to or from glandular cells [35]; 4) activities analogous to those of glandular cells possessing small granules [13, 36]; 5) stem cell-like activity [37] or a nursing effect on glandular cells [17]. No conclusion can be drawn from the present immunohistochemical observation as regards the intrinsic features of folliculo-stellate cells. However, the reported findings suggest that the S-100 protein exerts an effect on prolactin, gonadotropin, and ACTH secretion, as based on available evidenceof the following phenomena: increased prolactin-secreting cells (data not shown), as well as decreased gonadotrophs in chronic alcoholic patients [24]; S-100 protein enhancement of rat hypophyseal prolactin secretion *in vitro* [18]; and the fact that S-100 cells and ACTH-producing cells form the human pars intermedia [7].

Acknowledgments. We would like to express our sincere gratitude to Professor Demon C. Herbert (Professor in the Department of Cellular and Structural Biology, The University of Texas Health Science Center at San

Antonio, TX, USA) for critically reviewing this manuscript.

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