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Geriatr Gerontol Int 2011; **11:** *114–118*

CASE REPORT

Usefulness of ^{18}F-fluorodeoxyglucose positron emission tomography for diagnosis of asymptomatic giant cell arteritis in a patient with Alzheimer's disease

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It is often difficult to diagnose disease in elderly patients, in particular those with dementia, who do not present with typical symptoms. This report describes our experience of an elderly patient (an 83-year-old woman) who presented with a chief complaint of memory loss, showed a marked inflammatory response, and was diagnosed with large-vessel giant cell arteritis (GCA) on the basis of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) findings. She had no symptoms typical of GCA including jaw claudication, visual field defect and heavy headed feeling. Corticosteroid therapy resulted in a trend toward improvement in the inflammatory response and then she first recognized that she might have experienced slight dull headache before treatment of GCA. This was probably because this patient had large-vessel GCA, which produces a few symptoms in the head and neck, and because she had Alzheimer's disease and could not accurately describe her symptoms. Our experience suggests the usefulness of FDG-PET for the diagnosis of GCA, particularly in elderly patients without typical symptoms. **Geriatr Gerontol Int 2011; 11: 114–118.**

Keywords: Alzheimer's disease, arteritis, inflammation, positron emission tomography.

Introduction

In daily clinical practice, physicians sometimes encounter elderly patients who do not have typical symptoms of a disease as young patients do. Elderly patients with dementia cannot describe their symptoms accurately, which often makes diagnosis more difficult. Patients with giant cell arteritis (GCA) are characterized by jaw claudication, diplopia and headache,[1] but elderly patients often do not have these symptoms. It is reported that ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) is effective for the diagnosis of systemic inflammatory disease.[2,3] Here, we report our

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Table 1 Blood data on admission

Parameter	Value	Parameter	Value	Parameter	Value
WBC	5400/μL	γ-GTP	22 IU/L	Fe	13 μg/dL
RBC	336 × 10^4/μL	CK	29 IU/L	UIBC	193 μg/dL
Hb	8.7 g/dL	ChE	162 IU/L	Ferritin	107 ng/mL
Ht	28.0%	AMY	111 IU/L	IgG	2200 mg/dL
MCV	83 fL	Lipase	33 IU/L	IgA	382 mg/dL
MCH	25.9 pg	BUN	12 mg/dL	IgM	81 mg/dL
MCHC	31.1%	Cre	0.69 mg/dL	TSH	0.419 μU/mL
Plt	53 × 10^4/μL	UA	4.3 mg/dL	fT3	2.2 pg/mL
Reticulocytes	0.7%	Na	139 mEq/L	fT4	1.27 ng/dL
PT%	76.5%	K	3.9 mEq/L	Vitamin B_1	55 ng/mL
TP	7.5 g/dL	Cl	104 mEq/L	Vitamin B_{12}	>1500 pg/mL
T-Bil	0.5 mg/dL	Ca	8.3 mg/dL	Folic acid	6.8 ng/mL
AST	15 IU/L	P	3.4 mg/dL	CRP	7.30 mg/dL
ALT	8 IU/L	Glu	170 mg/dL	ESR	>100 mm/h
ALP	255 IU/L	TG	47 mg/dL	HBsAg	(–)
LDH	139 IU/L	HDL-C	42 mg/dL	HCV-Ab	(–)
		LDL-C	56 mg/dL		

ALP, alkaline phosphatase; ALT, alanine transaminase; AMY, amylase; AST, aspartate transaminase; BUN, blood urea nitrogen; Ca, calcium; Cl, chloride; ChE, choline esterase; Cre, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Fe, iron; fT3, free triiodothyronine; fT4, free thyroxine; Glu, glucose; Hb, hemoglobin; HBsAg, hepatitis B surface antigen; HCV-Ab, hepatitis C virus antibody; HDL-C, high-density lipoprotein cholesterol; Ht, hematocrit; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; K, potassium; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; P, phosphorus; Plt, platelet count; PT, prothrombin time; RBC, red blood cell count; γ-GTP, γ-glutamyl transpeptidase; T-Bil, total bilirubin; TG, triglyceride; TP, total protein; UA, uric acid; UIBC, unsaturated iron binding capacity; WBC, white blood cell count.

experience in a patient with GCA who showed a marked inflammatory response during tests for cognitive function, in whom the cause of inflammation was effectively diagnosed by FDG-PET.

Case report

An 83-year-old woman attended the outpatient department of our hospital with a chief complaint of memory loss. Her memory impairment had begun 1 year earlier and slowly progressed. From 4 months earlier, she had refused to take a bath occasionally. From 3 months earlier, she had begun to say or ask the same thing many times, and forgot appointments to meet her friends more frequently. She was aware of her memory loss. She could no longer manage money and began to dislike going shopping in the neighborhood. During the course of observation, she did not have such symptoms as jaw claudication, visual field defect, headache or numbness of the upper limbs. She had lost 3 kg in 1 month prior to admission, and was found to have a significant inflammatory response on blood tests, and was admitted to our department for further evaluation of cognitive impairment in June 2008.

She had no particular medical history. Her sister had a history of pituitary adenoma without neurological disorder or dementia. She had no family history of collagen disease. She did not smoke, and drank alcohol only on social occasions. She had no history of allergy and had never been abroad. She had been educated for 8 years. She had worked as an accountant until the age of 60 years. She lived with her daughter's family.

She was 146.0 cm tall and weighed 39.8 kg, with a body mass index of 17.3 kg/m^2. Temperature was 37.1°C, and pulse was regular (89 b.p.m.). Blood pressure was 96/56 mmHg (left upper limb) and 108/56 mmHg (right upper limb). She had clear consciousness. No arterial bruit was heard in the carotid arteries. The temporal arteries were non-tender on palpation. The palpebral conjunctivae were pale, but the bulbar conjunctivae were not icteric. Funduscopic findings were normal. Superficial lymph nodes were not palpable and the thyroid gland was not enlarged. There were no abnormal findings in the thoracoabdominal region. Examination of the skin revealed no redness or rash. The limbs were not edematous. There was no arthralgia. Neurological findings were normal.

Blood data are presented in Table 1. Urine was negative for occult blood and protein, and many white cells were observed in the urinary sediment. Chest radiograph and electrocardiogram were normal.

Neuropsychological tests showed cognitive deterioration, with a Mini-Mental State Examination (MMSE) score of 20 points, and a revised Hasegawa Dementia Scale (HDS-R) score of 16 points. Specifically, she did not score high in delayed recall, calculation, orientation and verbal recall. The 10-word recall test and the Rey–Osterrieth complex figure test also indicated a significant decline in delayed recall. She was able to recall 5 digits in the same order as they had been presented and 3 digits in the reverse order. She took 210 s to finish the trail making test part A (mean: 151 s in healthy persons), and part B was discontinued because she did not understand the task. She was thus found to have reduced overall cognitive function with memory impairment and disturbance of attention.

Cranial magnetic resonance imaging (MRI) showed diffuse cerebral atrophy on T_1-weighted images; particularly, the Sylvian fissure and the inferior horns of the lateral ventricles were dilated, while there was marked atrophy in the medial parietal and temporal lobes. T_2-weighted images and fluid-attenuated inversion recovery images showed lesions deep in the white matter and a high-signal-intensity area in the paralateral ventricles, appropriate for her age. Brain perfusion scintigraphy (^{123}I-iodoamphetamine single photon emission computed tomography) showed reduced blood flow in the medial temporal lobes, parietal-temporal association area and precuneus. Cerebrospinal fluid was colorless and transparent and showed a slightly increased protein level (65 mg/dL), with no increase in cell count (1/μL) and a normal glucose level (63 mg/dL) and blood glucose level (94 mg/dL). There was no obvious inflammatory response, with immunoglobulin (Ig)G index of 0.05 and negative test results for anti-herpes IgM and IgG antibodies. Phosphorylated tau protein level was 50.03 pg/mL (reference value: ≤31.3 pg/mL), and amyloid β1-42 level was 254.31 pg/mL (reference value: ≥1005 pg/mL), supporting the diagnosis of Alzheimer's disease.

With regard to inflammatory responses, the patient had a persistent slight fever after admission, but did not experience any obvious symptoms including apparent appetite loss. She had lost approximately 3 kg during 1 month before admission. She had shown a prolonged inflammatory response since attendance at our outpatient department, and had normocytic normochromic anemia, decreased Fe, and increased ferritin, suggesting chronic inflammation. The patient also underwent investigation for systemic diseases, including infections, malignant diseases and collagen diseases.

Urinalysis on admission showed pyuria, and she was treated with 200 mg/day of levofloxacin under a diagnosis of urinary tract infection. The urinary findings improved, while the inflammatory response did not. Plain computed tomography (CT) of the chest and abdomen, upper and lower gastrointestinal endoscopy, and transthoracic echocardiography were performed to search for the site of inflammation, but failed to detect any obvious findings. Blood cultures were negative.

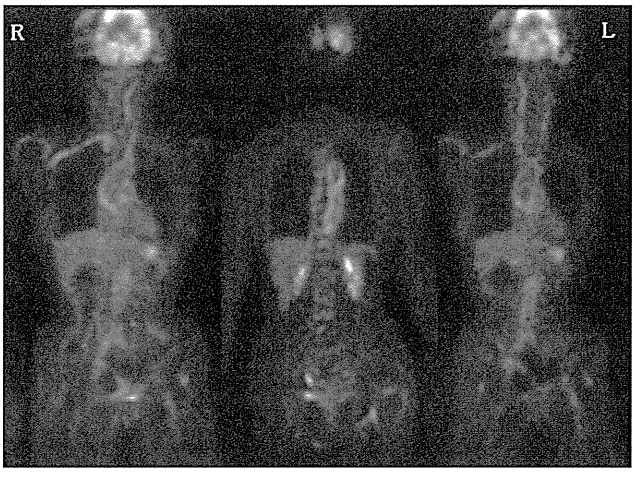

Figure 1 ^{18}F-Fluorodeoxyglucose positron emission tomography (FDG-PET) demonstrated increased glucose uptake in the ascending aorta, both carotid arteries, both subclavian arteries, and from the descending aorta to both common iliac arteries.

To search for collagen diseases, blood tests were performed and showed an elevated rheumatoid factor titer of 1:25 and an increased anti-cyclic citrullinated peptide antibody level of 277.0 U/mL. Early rheumatoid arthritis was suspected, but the patient did not have any joint symptoms, and radiography of the joints of the whole body did not show any findings suggestive of rheumatism.

Therefore, FDG-PET (Fig. 1) was performed to identify the site of inflammation. This examination showed increased glucose uptake in the ascending aorta, both carotid arteries, both subclavian arteries, and from the descending aorta to both common iliac arteries, raising the suspicion of aortitis syndrome. Contrast-enhanced CT of the chest and abdomen (Fig. 2) showed thickening of the wall of the thoracoabdominal aorta and delayed contrast enhancement of this part of the aorta. Ultrasonography of the superficial temporal arteries revealed a halo in both superficial temporal arteries and stenosis/occlusion of both frontal branches and both occipital branches. Biopsy of the left superficial temporal artery showed giant cell and lymphocytic infiltration of the arterial wall, leading to the diagnosis of GCA (Fig. 3).

Although the patient had extensive vasculitis, treatment was initiated with 20 mg of oral prednisolone because she did not have any lesions in the ocular fundi, and taking her age into consideration. Because she had a reduced bone mass (59% and 84% of that of the young adult mean measured in the forearm and a lumbar vertebra, respectively), oral bisphosphonate was also administrated for the prevention of steroid-induced osteoporosis. Corticosteroid therapy resulted in a trend

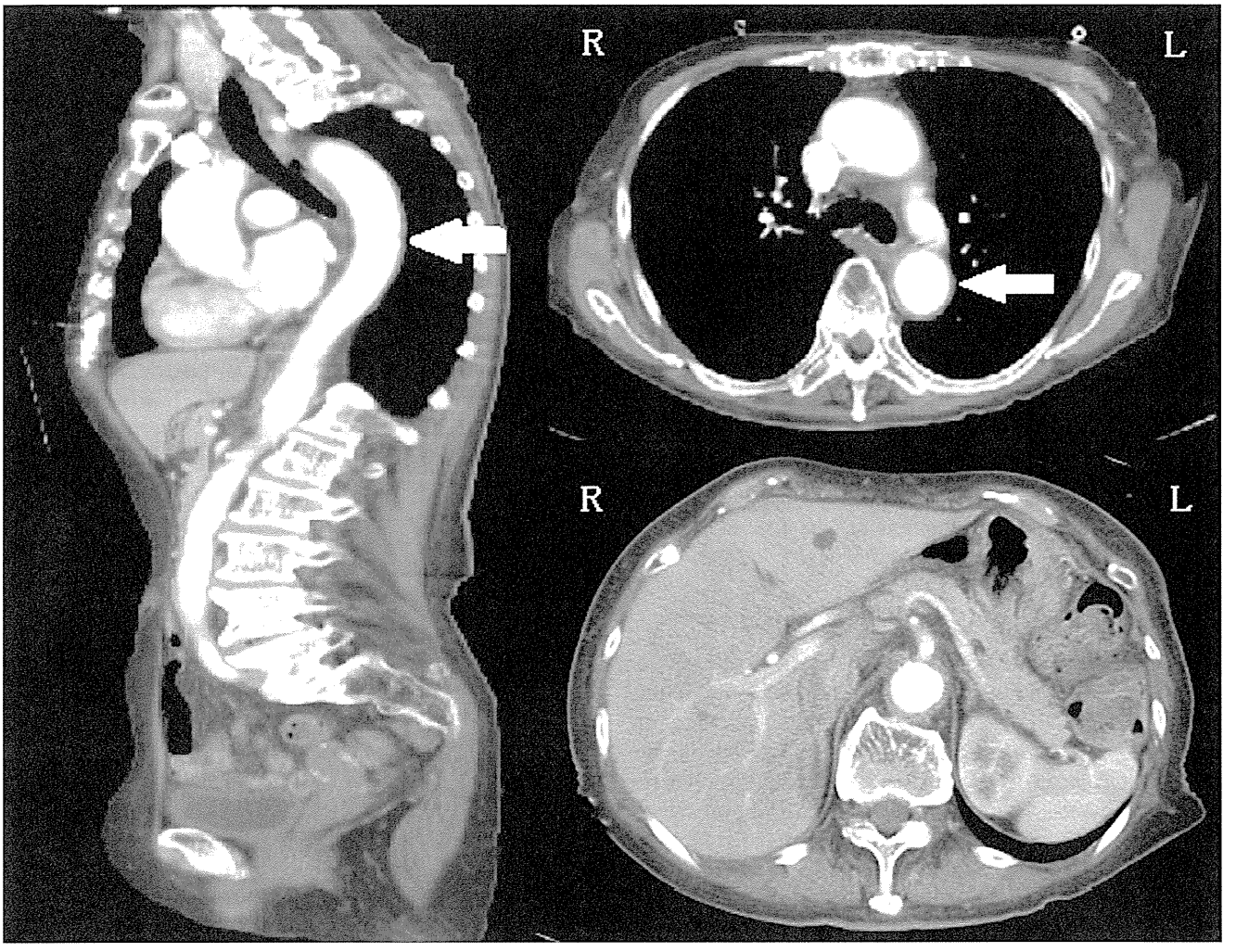

Figure 2 Contrast-enhanced computed tomography (CT) image. Contrast-enhanced CT of the chest and abdomen showed thickening of the wall of the thoracic descending aorta to the abdominal aorta and both common iliac arteries, and delayed contrast enhancement of these arteries. None of the aortic branches was stenosed.

toward improvement in the inflammatory response. The patient did not complain of her heavy headed feeling or any similar symptoms before treatment, but after successful treatment of GCA, she first recognized that she might have experienced slight dull headache. On day 29 of corticosteroid therapy, blood tests indicated improvement of the inflammatory response, with a negative result for C-reactive protein and an erythrocyte sedimentation rate of 21 mm after 30 min and 52 mm after 60 min. Accordingly, the dose of prednisolone was reduced to 18 mg. On day 32 of corticosteroid therapy, contrast-enhanced CT demonstrated reduced thickening of the aortic wall and reduced contrast enhancement. Neuropsychological tests were performed again on days 10 and 29 after initiation of oral corticosteroid therapy, but revealed no improvement (day 10: MMSE, 21 points; HDS-R, 13 points. Day 29: MMSE, 17 points; HDS-R, 12 points). The patient subsequently made favorable progress and was discharged.

Discussion

Giant cell arteritis normally occurs in patients aged 50 years or older. It is a granulomatous angiitis involving the aorta and its major branches. According to the classification proposed by the American College of Rheumatology,[4] GCA should be diagnosed if at least three of the following five criteria are present: (i) age at disease onset of 50 years or more; (ii) new onset of localized headache; (iii) temporal artery tenderness to palpation or decreased pulsation; (iv) elevated erythrocyte sedimentation rate (≥50 mm/h); and (v) biopsy specimen with an artery showing necrotizing vasculitis characterized by a predominance of monocytes, or granulomatous change with multinucleated giant cells.

Because our patient did not complain of headache at first, FDG-PET, which was performed to identify the site of inflammation, played an important role in diagnosis.

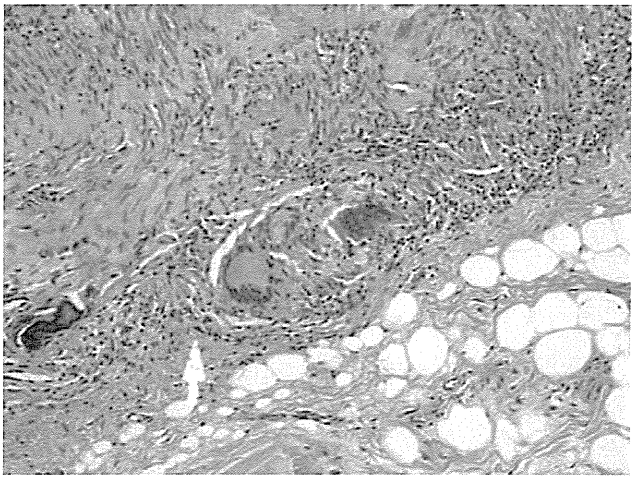

Figure 3 Histological findings of temporal artery. Biopsy of the left superficial temporal artery showed multinucleated giant cell and lymphocytic infiltration of the internal elastic lamina of the arterial wall (hematoxylin–eosin, original magnification ×200).

Brack *et al.* reported that GCA can be divided into two groups: classic cranial GCA and large-vessel GCA. They mentioned that headache was the most frequent symptom for cranial GCA (42%), but not for large-vessel GCA.[5] According to them, vasculitis is present around the aorta in large-vessel GCA. Temporal artery biopsy findings were negative in 42% of patients with large-vessel GCA, and many patients had ischemic disorders of the upper limbs (arterial bruit, 80%; pain on movement, 78%; difference in blood pressure measured in both arms, 58%) as initial symptoms, while only 10% of patients had headache as an initial symptom. They also reported that the time from disease onset to diagnosis was significantly longer in patients with vasculitis only involving the cranial arteries.[5] Although there has been a report of a patient without headache in whom GCA was diagnosed on the basis of elevated glucose uptake in the aorta detected by FDG-PET, as was the case in our patient,[6] thickening of the vessel wall shown by CT or MRI is also known to be a useful finding.[7,8] The clinical course in our patient was consistent with that of large-vessel GCA, and she may have had fewer symptoms in the head and neck compared to those in the trunk and upper limbs. In addition, she may not have been able to accurately express her symptoms because of Alzheimer's disease.

Some patients with GCA also have cerebral infarction or transient cerebral ischemia resulting from vasculitis. In one study, 3% of patients with GCA had psychiatric symptoms, including depression,[9] but it is not clear whether ischemia was involved in the mechanism. While there is a report of GCA in a patient with cognitive impairment that was improved by corticosteroid therapy,[10] our patient did not show any change in cognitive function after initiation of corticosteroid therapy, and thus there seems to be no relation between GCA and cognitive impairment. The decline of the score of MMSE was considered that it might be the effect of corticosteroids or hospitalization or progression of Alzheimer's disease. Our patient's course was also consistent with the diagnosis of Alzheimer's disease.

As detailed above, GCA should be positively differentiated when elderly patients have an inflammatory response, even if they do not have typical symptoms such as headache. In our patient, GCA was effectively diagnosed by evaluation of blood vessels by imaging, including FDG-PET and contrast-enhanced CT. Because of its high sensitivity in an active inflammatory state,[2] FDG-PET might become a powerful diagnostic tool in the management of large-vessel inflammation.

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Geriatr Gerontol Int 2012; **12:** *131–139*

ORIGINAL ARTICLE: BIOLOGY

Role of the mTOR complex 1 pathway in the *in vivo* maintenance of the intestinal mucosa by oral intake of amino acids

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Aim: Oral intake of nutrients is often compromised in elderly, multimorbid patients, but parenteral nutrition causes intestinal atrophy and impairs intestinal function. To uncover the molecular mechanisms by which amino acids are involved in intestinal atrophy and recovery, we studied whether the rapamycin-sensitive mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) pathway is involved in this process.

Methods: C57BL/6N mice were fed a glucose solution alone, glucose solution with amino acids or normal chow diet for various lengths of time. Intestinal sections were prepared from these mice and the villus height and villus density were quantified. As a readout for the mTORC1 pathway, the phosphorylation of the ribosomal S6 protein (S6) was analyzed by immunostaining and immunoblotting. To confirm the role of the mTORC1 pathway, the inhibitory effect of a specific mTOR inhibitor, rapamycin, was examined.

Results: Inducing fasting in mice fed only glucose caused time-dependent intestinal mucosal atrophy, whereas supplementation with amino acids protected the intestinal mucosa from atrophy. Phosphorylation of S6 decreased in the intestinal mucosa of mice fed only glucose, whereas supplementation with amino acids increased S6 phosphorylation. Importantly, intraperitoneal injection of rapamycin attenuated the protective effect of amino acids on the intestinal mucosa in a pattern consistent with the decrease of S6 phosphorylation.

Conclusions: These results indicate that the mTORC1 pathway plays a crucial role in the *in vivo* maintenance of the intestinal mucosa by the oral intake of amino acids. **Geriatr Gerontol Int 2012; 12: 131–139.**

Keyword: amino acids, enteral nutrition, intestinal mucosa, mTOR, rapamycin.

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Introduction

Oral intake of nutrition is often compromised in elderly, multimorbid patients with severe neurological dysphagia, depression or dementia. However, parenteral nutrition causes intestinal atrophy and impairs intestinal function increasing the risk of infection caused by

bacterial translocation through the intestinal membrane. The European Society for Clinical Nutrition and Metabolism guidelines recommend enteral nutrition to maintain or improve nutritional status in geriatric patients.[1] The small intestine is the primary organ responsible for the terminal digestion and absorption of dietary nutrients. Many dietary factors, including glucose and amino acids, are required nutrients and act as regulators of the intestinal mucosa. It is important to establish a clinically relevant model of intestinal atrophy induced by starvation. Intestinal atrophy is characterized by anatomical changes in the intestine including decreased villus height, villus surface area, and epithelial cell number. Several studies have shown that certain amino acids, such as leucine, arginine, and glutamine, play critical roles in the maintenance of the intestine, although the molecular mechanisms appear to be diverse and complex.[2–4]

Recent evidence indicates that the signals derived from nutrients play important roles in the control of cellular activities. The mechanistic target of rapamycin (mTOR), responds to changes in amino acid availability and regulates cell growth and proliferation.[5] TOR was discovered in yeast as a target of the macrolide rapamycin, which is used widely in clinical fields as an immunosuppressant and an anticancer drug. mTOR signals through two distinct multiprotein complexes called mTOR complex (mTORC)1 and mTORC2 which are sensitive and insensitive to rapamycin, respectively.[5] mTORC1 senses the changes in the ambient nutritional environment, including changes in amino acid concentration, but the upstream mechanism underlying the regulation of mTORC2 is unclear. mTORC1 contains a scaffold protein, raptor, which plays an essential role in proper substrate recognition of the translational regulators, eukaryotic initiation factor-4E binding protein 1 (4E-BP1) and p70 S6 kinase (p70S6K).[6–8] Under reduced nutrient conditions, such as limited amino acid availability, protein synthesis is downregulated at least in part because of reduced mTORC1 activity, which decreases p70S6K activity and induces 4E-BP1 dephosphorylation.[9,10] Thus, mTORC1 regulates protein synthesis and cell growth by sensing changes in the ambient nutritional environment, including changes in amino acid availability. This raises the possibility that the mTORC1 pathway is an integral part of the regulation of the intestinal mucosa by amino acids.

To understand the roles of amino acids in the prevention of intestinal atrophy and the molecular mechanisms by which amino acids are involved in pathological changes and recovery, we studied whether the rapamycin-sensitive mTORC1 pathway is activated by amino acids in the intestinal mucosa and whether activation of the mTORC1 pathway helps prevent mucosal atrophy *in vivo*.

Methods

Materials

Male 8–9-week-old C57BL/6N mice were maintained in the Institute for Experimental Animals, Kobe University School of Medicine. All animals were handled under the Guidelines for Animal Experimentation of Kobe University School of Medicine.

In the present study, we examined five dietary groups: a glucose-only diet (GlcoD) group; an amino acid diet (AAD) group; a normal chow diet (NCD) group; control diet (ContD) group; and glutamine diet (GlnD) group. Distilled 10% glucose solution was used as the GlcoD. The AAD comprised the 10% glucose solution supplemented with a standard amino acid solution (0.25% alanine, 0.45% arginine, 0.1% aspartic acid, 0.1% cysteine, 0.1% glutamic acid, 0.35% histidine, 0.9% isoleucine, 1.4% leucine, 0.5% lysine, 0.3% methionine, 0.5% phenylalanine, 0.3% proline, 0.3% serine, 0.35% threonine, 0.25% tryptophan, 0.05% tyrosine, and 1.00% valine). The NCD comprised CLEA Rodent Diet CE-2 (CLEA Japan, Tokyo, Japan) with distilled 10% glucose solution. The ContD comprised NCD with a standard amino acid solution. The GlnD comprised the standard amino acid solution with 10% glucose and 2% glutamine. Mice had free access to the normal chow diet or the indicated diets ad libitum.

Tissue collection

All mice were killed by cervical luxation. The abdomen was opened, and the gastrointestinal tract was removed from the duodenum to the proximal end of the ileocecum. The removed small intestinal segment was laid in sigmoid form, and the middle section was divided into two parts; the first half was considered the jejunum and the second half was considered the ileum. A 3cm section was obtained from the proximal end of the jejunum and rinsed in cold saline. The first 1 cm of the section from proximal end of the jejunum was pinned flat onto corkboard and then placed in 10% buffered formalin for morphological analysis. The next 2 cm section of jejunum was frozen in liquid nitrogen for immunoblot analysis.

Immunoblot analysis

Anti-phospho-S6 ribosomal protein (Ser235/236) and anti-S6 ribosomal protein antibodies were purchased from Cell Signaling Technologies (Danvers, MA, USA). The frozen intestines were minced and homogenized using a Dounce tissue grinder (Wheaton, Millville, NJ, USA) on ice in ice-cold buffer C (20 mmol/L Tris-HCl, pH 7.4, 120 mmol/L NaCl, 1 mmol/L

ethylenediaminetetraacetic acid (EDTA), 5 mmol/L ethylene glycol bis (2-aminoethylether) tetraacetic acid (EGTA), 50 mmol/L β-glycerophosphate, 50 mmol/L NaF, 0.3% 3- (3-cholamidepropyl) dimethylammonio-1-propanesulphonate (CHAPS), 1 mmol/L dithiothreitol, 4 μg/mL leupeptin, and 4 μg/mL aprotinin) and then centrifuged at 12 000 × *g* for 30 min at 4°C. The supernatant was collected and the protein concentration was measured using the Bio-Rad Protein Assay Reagent S (Bio-Rad Laboratories, Hercules, CA, USA). The same amount of supernatant was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was immunoblotted with an appropriate first antibody and visualized using the enhanced luminol-based chemiluminescent method. Where indicated, the first antibody was stripped from the membrane, and the membrane was reused for the second immunoblot.

Histological analysis

Samples were fixed and embedded in paraffin, sectioned at a thickness of 5 μm, and stained with hematoxylin and eosin (HE). The villus height was measured from the villus top to the muscularis mucosae. The villus area, including the area from the villus top to the muscularis mucosae, was photographed with a digital camera and analyzed using ImageJ (Research Services Branch, National Institute of Mental Health, Bethesda, MD, USA). [11]. Five villus heights and five villus areas were measured from each section, and three sections from each mouse were analyzed. Villus density was calculated as the product of the number of villi and the villus area divided by the muscularis mucosa length. Immunostaining to identify phosphorylated S6 ribosomal protein was performed as described previously.[12]

Rapamycin administration

Mice were injected intraperitoneally with rapamycin (4 mg/kg or 20 mg/kg) dissolved in 0.2% sodium carboxymethylcellulose and 0.25% Tween 80 three times a week from 7 to 9 weeks of age.

Measurement of metabolic parameters

Blood was collected from the tail vein. Plasma glucose concentration was measured using the Glutest-AceR kit (Sanwa Kagaku Kenkyusho, Aichi, Japan). Serum albumin and insulin concentrations were measured using the A/G B-test (Wako, Osaka, Japan) and Ultra Sensitive Mouse Insulin ELISA Kit (Morinaga, Kanagawa, Japan), respectively.

Statistical analysis

Data are expressed as the means ± SD and were analyzed using the Mann–Whitney nonparametric *U*-test. Two-tailed *P* values < 0.05 were considered significant.

Results

Intestinal mucosal atrophy induced by food restriction

To explore the mechanism by which the intestinal mucosa is maintained by the oral intake of amino acids, we first tried to establish an *in vivo* system to examine the intestinal mucosal atrophy arising from food restriction in a mouse model. C57BL/6 mice were fed water containing 10% glucose alone (GlcoD) or standard rodent diet with distilled 10% glucose solution (NCD) for various lengths of time, intestinal sections were then prepared from these mice (Fig. 1a). HE staining of the intestinal sections showed a progressive decrease in the heights and the density of intestinal villi in a time-dependent manner. Quantification of the intestinal villi demonstrated that the villus height decreased progressively from 97.9% ± 2.7% in mice fed the NCD to 78.6% ± 1.6% after 48 h of the GlcoD and to 65.8% ± 3.2% after 96 h of the GlcoD (Fig. 1b). Similarly, villus density decreased progressively from 93.5% ± 8.3% in mice fed the NCD to 53.0% ± 3.6% after 48 h of the GlcoD and to 46.8% ± 6.5% after 96 h of the GlcoD (Fig. 1c). These observations are consistent with the idea that the turnover of the epithelial cells of the gastrointestinal tract occurs every 2–7 days under normal homeostasis.[13] Thus, feeding for 96 h was used in the following experiments.

Effect of oral amino acid intake on intestinal mucosal atrophy

Next, to explore the mechanism by which the intestinal mucosa is maintained by amino acids, mice were fed 10% glucose with or without a mixture of amino acids (GlcoD and AAD, respectively). As shown in Figure 2a, villus height and density were significantly lower in the mice fed the GlcoD for 96 h compared with the ContD. By contrast, the AAD partially protected the intestinal mucosa from atrophy: villus heights were 55.6% ± 7.6% in the GlcoD group and 68.9% ± 5.1% in the AAD group, and the villus densities were 37.2% ± 4.0% and 59.4% ± 11.5%, respectively (Fig. 2b,c). Body weights were similar in mice fed the GlcoD to those fed the AAD, but both were significantly lower than in mice fed the ContD (Table 1). Glucose and albumin concentrations did not differ significantly between the three groups. Insulin concentration was significantly higher in the mice fed the ContD than in mice fed the GlcoD or the AAD.

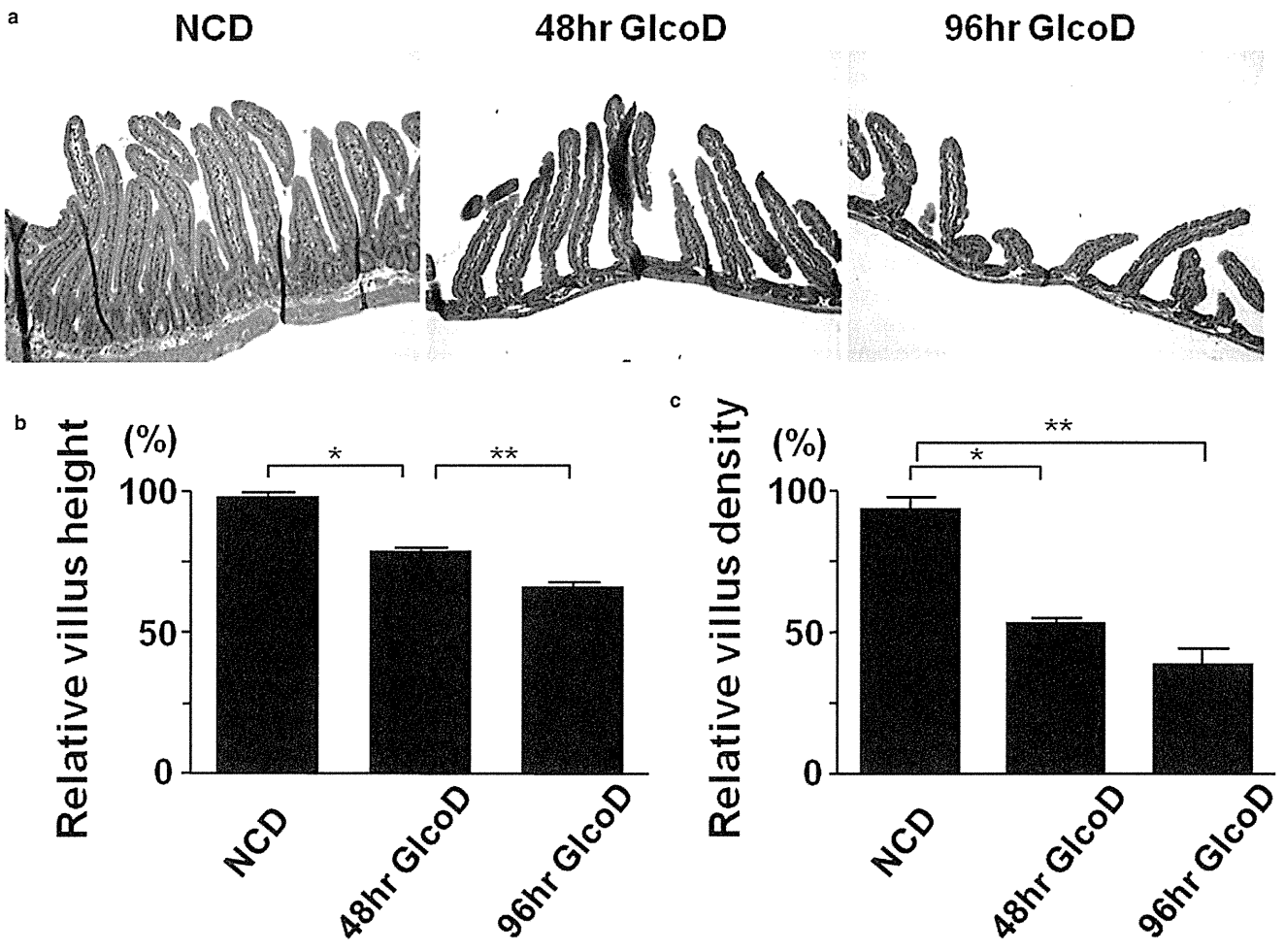

Figure 1 Intestinal mucosal atrophy caused by food restriction in C57BL/6N mice. (a) Hematoxylin and eosin staining of representative jejunal sections from mice fed the glucose-only diet (GlcoD) or the normal chow diet (NCD) for the indicated times. (b, c) Villus height (b) and villus density (c) were measured as described in the Methods section. The value for the mice fed NCD was set to 100%, and the values in the other groups are expressed relative to the value in the mice fed the NCD. Data are the means ± SD of values from 4–6 (b, c) animals from each group. $^{*}P < 0.05$; $^{**}P < 0.01$.

Because the AAD used in the experiments mentioned above did not include glutamine, we next examined whether the addition of glutamine improved the protective effect of the AAD. Glutamine added to the diet (GlnD) did not give any additive effect on protecting the intestinal mucosa beyond that induced by the AAD; the villus heights were 103.4% ± 4.0% in mice fed the AAD and 99.7% ± 13.1% in mice fed the GlnD, and the villus densities were 94.8% ± 13.3% and 88.5% ± 16.0%, respectively (Fig. 3a–c).

Effect of oral amino acid intake on the mTORC1 pathway in the intestinal mucosa

We next examined the possible involvement of the mTORC1 pathway by analyzing phosphorylation of the S6 ribosomal protein (S6), a well-characterized downstream marker of the mTORC1 pathway. Immunostaining of sections of the intestinal membranes with the anti-phospho-S6 antibody showed more abundant dark-staining cells in the mucosal areas than in the submucosal areas (Fig. 4a). Sections from the ContD group viewed under a high-power field demonstrated that S6 phosphorylation was most abundant in the crypt cells and to a lesser extent in enterocytes in the villi, whereas goblet cells showed poor immunoreactivity. Sections from mice fed the GlcoD showed less staining in the villus and crypt areas than did sections from mice fed the AAD, suggesting that the mTORC1 pathway was inactivated by feeding the GlcoD for 96 h. Immunoblotting of the intestinal lysates with the anti-phospho-S6 antibody confirmed that S6 phosphorylation was significantly lower in the intestine of mice fed the GlcoD than in samples from those fed the AAD (75.9% ± 27.9% vs. 129.6% ± 12.5%). These results suggest that the mTORC1 pathway is regulated in the intestinal mucosa by oral amino acid intake (Fig. 4b,c).

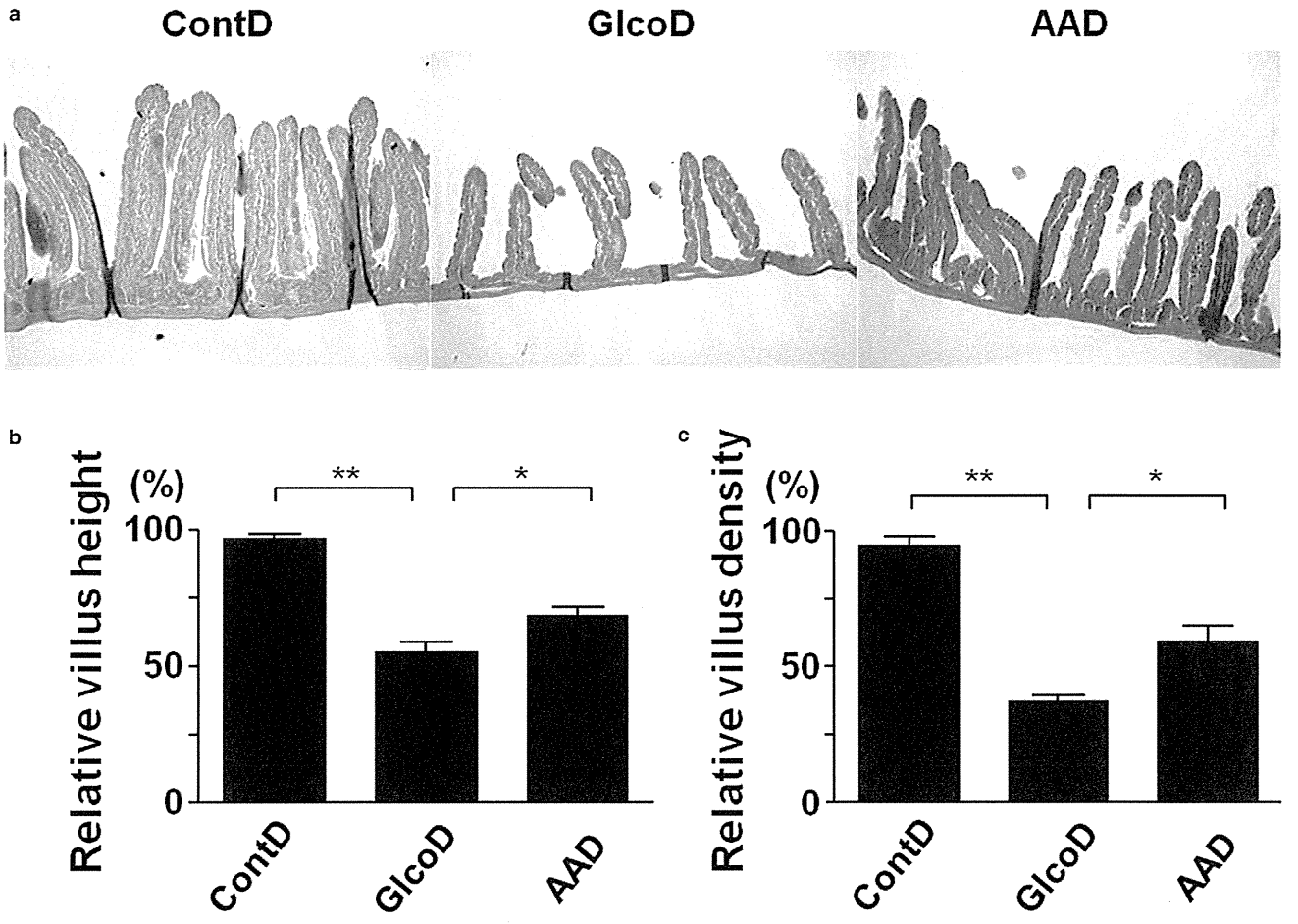

Figure 2 Prevention of intestinal mucosal atrophy by oral intake of amino acids. (a) Hematoxylin and eosin staining of representative jejunal sections from mice fed the control diet (ContD), the glucose-only diet (GlcoD), or the amino acid diet (AAD). (b, c) Villus height (b) and villus density (c) were measured as described in the Methods section. The value for the mice fed with ContD was set to 100% and the values in the other groups are expressed relative to this. Data are the means ± SD of values from 5–6 (b, c) animals from each group. $^{*}P < 0.05$; $^{**}P < 0.01$.

Table 1 Body weight and metabolic parameters

	Body weight (g)	Blood glucose (mg/dL)	Insulin (pg/mL)	Albumin (g/dL)
Control diet	22.1 ± 1.9***	131.6 ± 29.7	0.7 ± 0.3*	3.7 ± 0.6
Glucose-only diet	19.0 ± 1.9	133.7 ± 33.0	0.3 ± 0.1	3.9 ± 1.0
Amino acid diet (AAD)	17.9 ± 1.3	113.7 ± 30.6	0.4 ± 0.1	4.4 ± 0.8
Glutamine diet	17.9 ± 0.9	97.5 ± 11.9	0.5 ± 0.2	4.9 ± 0.2
AAD with 4 mg/kg rapamycin	18.8 ± 0.9	138.6 ± 52.7	0.5 ± 0.2	3.7 ± 0.6
AAD with 20 mg/kg rapamycin	17.5 ± 0.8	162.8 ± 11.3**	0.6 ± 0.2*	5.1 ± 1.1

$^{*}P < 0.05$ vs. amino acid diet, $^{**}P < 0.01$ vs. amino acid diet, $^{***}P < 0.001$ vs. amino acid diet. Data are means ± SD of values from 5–12 animals from each group at 8 weeks.

Effect of rapamycin on the amino acid protection of the intestinal mucosa

To confirm the role of the mTORC1 pathway in the regulation of the intestinal mucosa, we examined the inhibitory effect of the mTOR inhibitor, rapamycin. Compared with vehicle treatment, rapamycin treatment significantly decreased villus height and density in mice fed the AAD; this effect was dose dependent (Fig. 5a). Villus height was 102.0% ± 4.8% in the vehicle-treated mice. This tended to decrease to 90.3% ± 10.2% in mice given 4 mg/kg rapamycin and was significantly

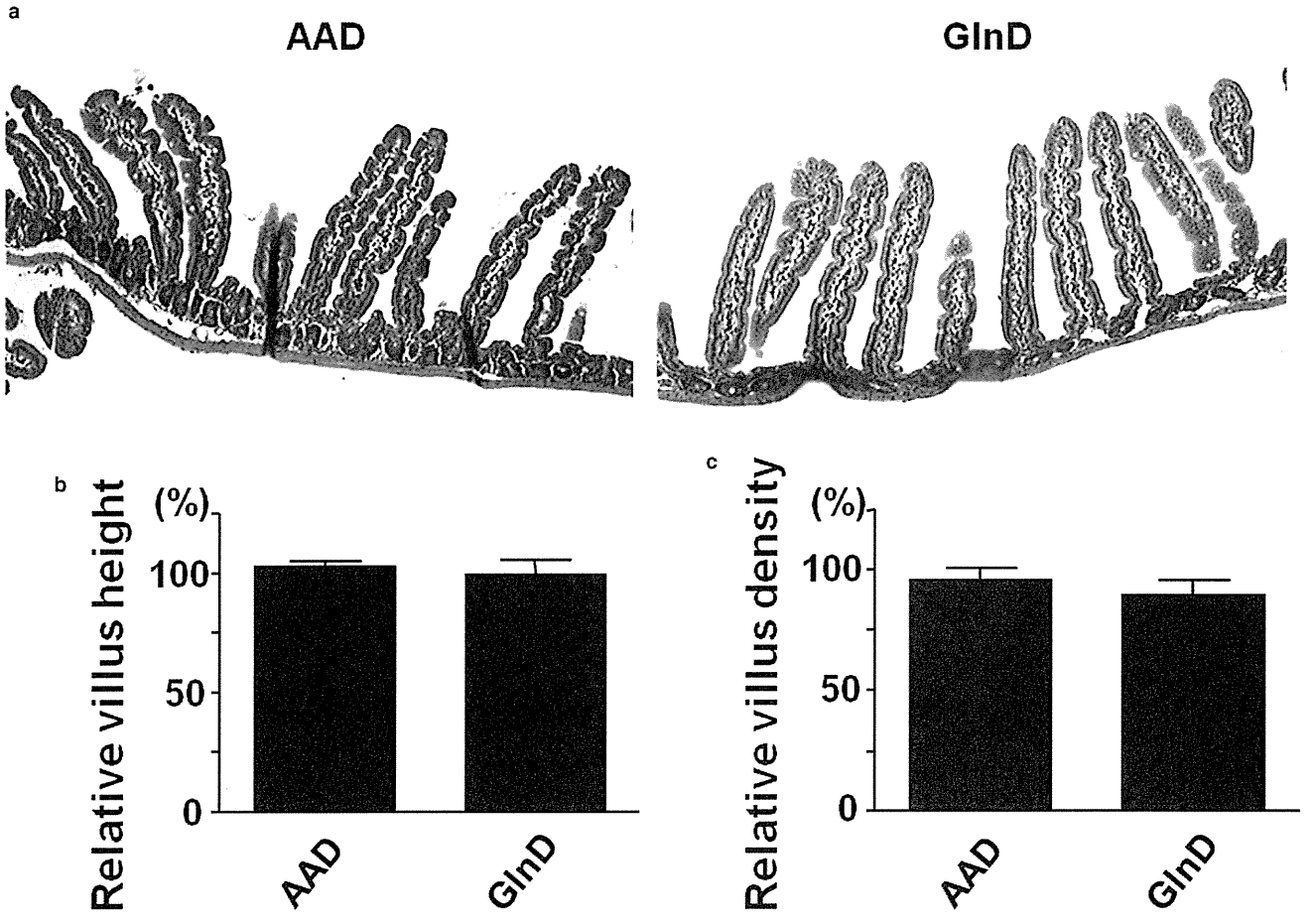

Figure 3 The effect of oral amino acids intake with or without glutamine on intestinal mucosal atrophy. (a) Hematoxylin and eosin staining of representative jejunal sections from mice fed the glutamine diet (GlnD) or the amino acid diet (AAD). Each diet is described in the Materials section. (b, c) Villus heights (b) and villus densities (c) were measured as described in Methods. The value for the mice fed AAD was set to 100% and the value the GlnD group is expressed relative to this. Data are the means ± SD of values from 6 (b, c) animals from each group.

decreased to 85.7% ± 8.9% in mice given 20 mg/kg rapamycin (Fig. 5b). Similarly, villus density was 97.3% ± 16.6% in the vehicle-treated mice, 80.5% ± 18.8% in mice given 4 mg/kg rapamycin, 63.3% ± 21.2% in mice given 20 mg/kg rapamycin (Fig. 5c). Immunoblot analysis of the intestinal lysates with anti-phospho-S6 antibody confirmed that rapamycin treatment decreased the extent of S6 phosphorylation in the intestinal mucosa in a dose-dependent manner (135.1% ± 33.8% in the vehicle-treated mice, 95.7% ± 49.0% in mice given 4 mg/kg rapamycin, 77.7% ± 40.6% in mice given 20 mg/kg rapamycin; Fig. 6a,b).

Discussion

In the present study, oral intake of amino acids protected against intestinal atrophy induced by a diet of glucose alone, and this protection was concomitant with activation of the mTORC1 pathway, as judged by S6 phosphorylation. The mTOR inhibitor rapamycin attenuated the protective effects of amino acids against mucosal atrophy, indicating a critical role of the mTORC1 pathway in the protection against intestinal atrophy *in vivo*. We reported previously that intraperitoneal injection of 2 mg/kg rapamycin was sufficient to attenuate mTORC1 activity in pancreatic β-cells.[12] However, the same dose was insufficient for inhibiting mTORC1 activity in the intestinal mucosa. Mori *et al.* also showed that a higher concentration of rapamycin is needed to inhibit mTORC1 activity in the hypothalamus.[14] Thus, sensitivity to rapamycin appears to vary between tissues. Rapamycin treatment did not cause significant differences in body weight and albumin concentration, but blood glucose and insulin concentrations were significantly higher in mice treated with rapamycin at 20 mg/kg than in mice treated with rapamycin at 4 mg/kg and in untreated mice (Table 1). This is consistent with our previous report showing that blood glucose concentration was nearly 50 mg/dL

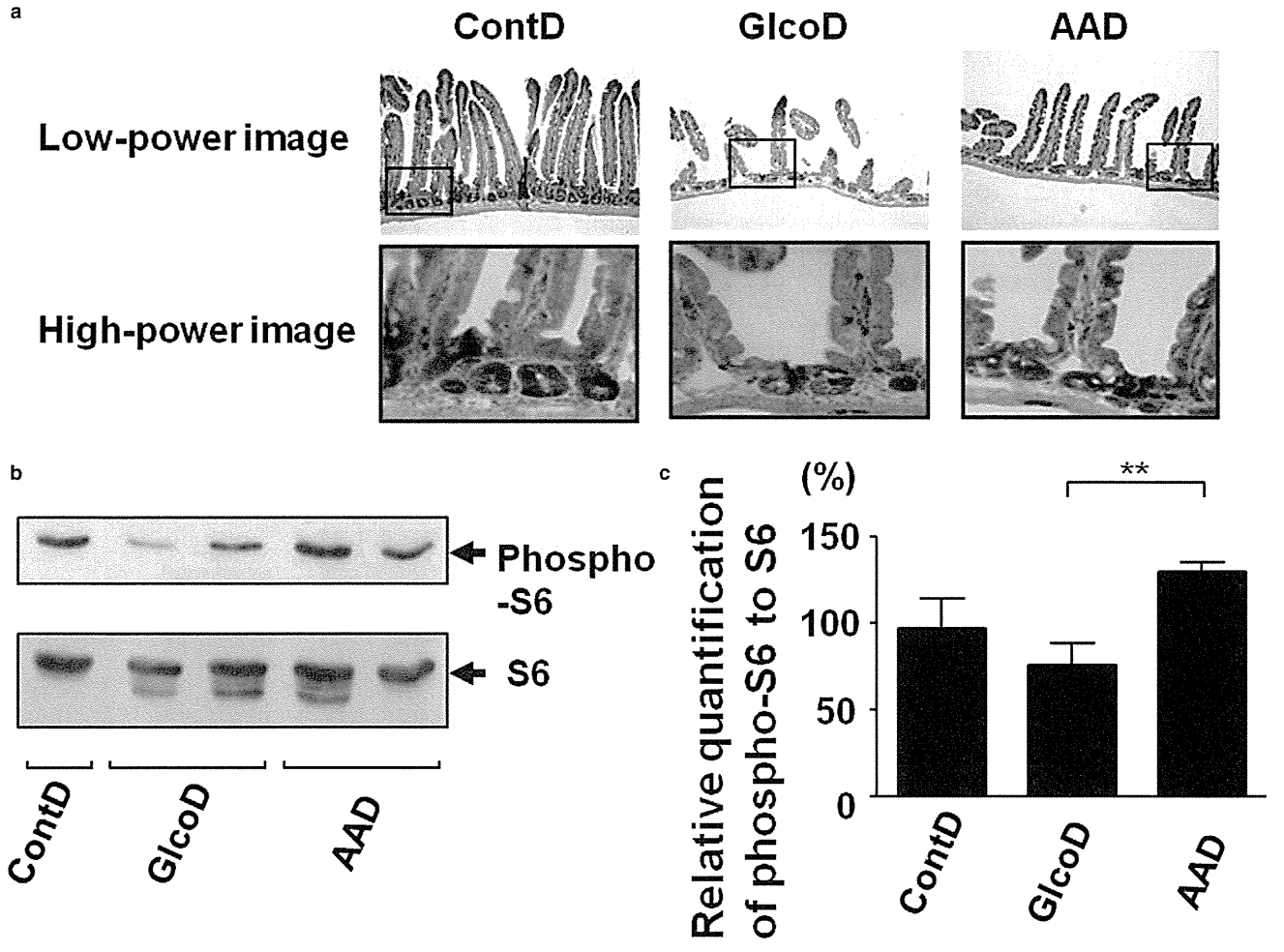

Figure 4 Phosphorylation of the S6 ribosomal protein in the intestinal mucosa. (a) Immunostaining with anti-phospho-S6 ribosomal protein (Ser235/236) antibody of representative jejunal sections from mice fed the control diet (ContD), the glucose-only diet (GlcoD), or the amino acid diet (AAD). The low- and high-power images are shown in the upper and lower lanes, respectively. (b) Intestinal mucosa was prepared from each group, and the same amount of protein extract was analyzed by immunoblotting with antibodies to phospho-S6 ribosomal protein (Ser235/236) or S6 ribosomal protein. (c) Relative quantification of phospho-S6 (Ser235/236) to S6. The immunoblots were scanned and the optical density of phospho-S6 ribosomal protein (Ser235/236) blot was divided by the optical density of the corresponding S6 ribosomal protein. Data are the means ± SD of values from six animals from each group. $^{**}P < 0.01$.

higher in rapamycin-treated mice than in untreated mice after glucose loading.[12] Because insulin concentration was higher in rapamycin-treated mice than in untreated mice, the higher glucose concentration appeared to be caused by impaired insulin resistance, although it remains to be clarified how rapamycin induces insulin resistance.

Although oral administration of the amino acid mixture clearly protected the intestinal mucosa from starvation-induced atrophy, the protective effect was less than that associated with the ContD. This supports the idea that other nutrients or ingredients in the ContD are also required to maintain the intestinal mucosa. For instance, dietary fiber and viscous diets appear to play important roles in stimulating cell turnover in the small intestine.[15,16] The extent of phosphorylation was more variable in mice fed the ContD than in those fed the GlcoD or the AAD. Nevertheless, S6 phosphorylation was consistently much higher in mice fed the AAD than in those fed the GlcoD. Thus, the effect of amino acids on the intestinal mucosa could be detected more easily under such simplified conditions. The variability of S6 phosphorylation in mice fed the ContD might be caused by other factors in this diet such as lipids or dietary fiber.

Several studies have shown that certain amino acids such as leucine, arginine, and glutamine play key roles in maintaining the intestine. However, we found no additive effect of glutamine on the protection of the intestinal mucosa by amino acids. Glutamine activates

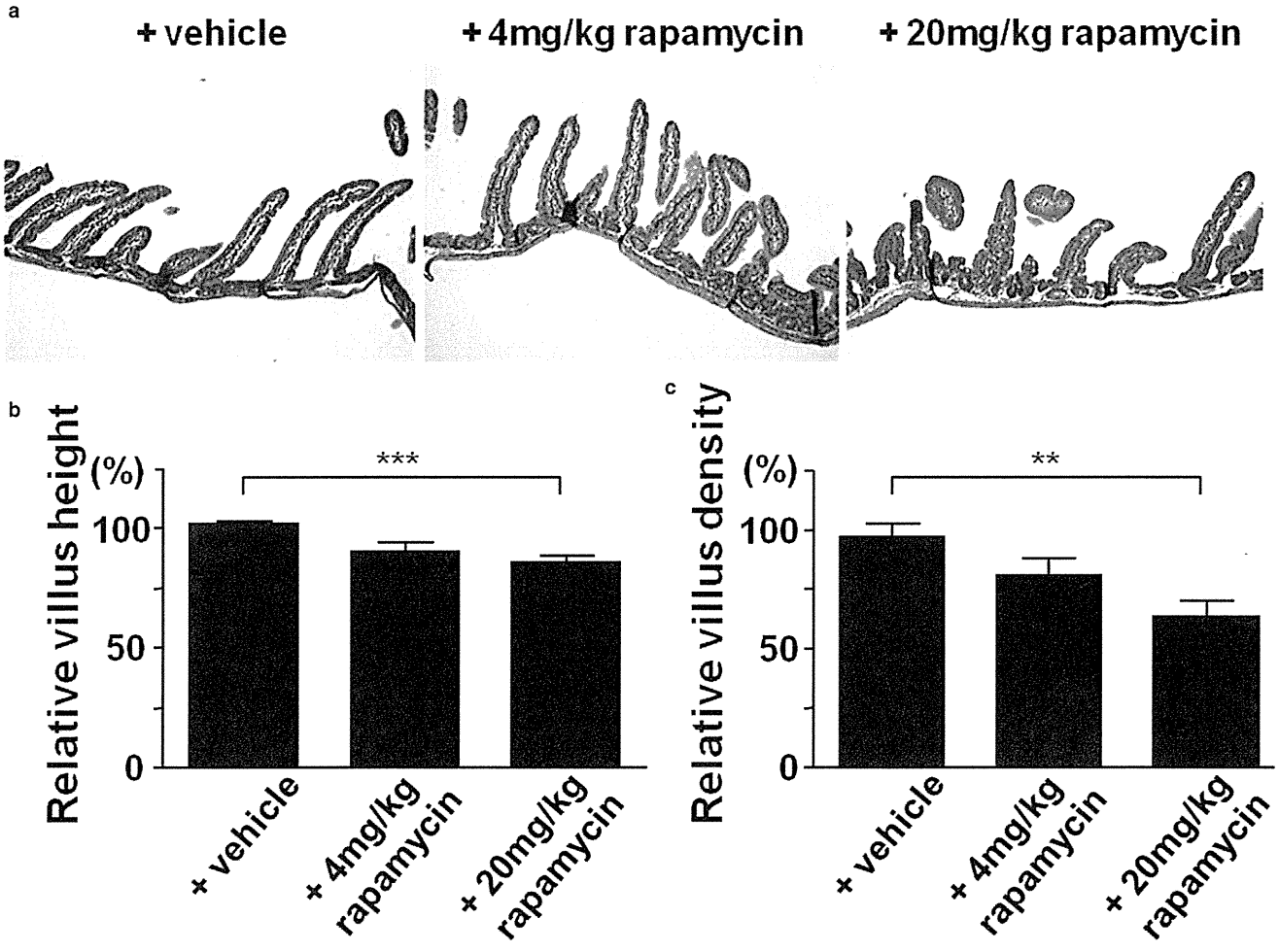

Figure 5 Suppression of the protective effect of amino acids against atrophy in the jejunal mucosa by rapamycin. (a) Hematoxylin and eosin staining of representative jejunal sections from mice fed the amino acid diet (AAD) treated with vehicle or the indicated concentrations of rapamycin. (b, c) Villus height (b) and villus density (c) were measured as described in the Methods section. The value for the group fed AAD treated with vehicle was set to 100%, and the values of the other groups are expressed relative to this value. Data are the means ± SD of values from 7–13 (b), and 7–12(c) animals from each group. $^{**}P < 0.01$; $^{***}P < 0.001$.

intestinal cell proliferation via mitogen-activated protein kinase in rat intestinal cell lines.[17] The bidirectional transport of leucine and glutamine is considered the rate-limiting step of mTORC1 activity,[18] and this observation supports the critical role of glutamine in the maintenance of the intestinal mucosa via mTORC1. On the other hand, glutamine induces autophagy and prevents apoptosis of the intestinal mucosa by inhibiting mTOR[19]. Glutamine inhibits the activation and/or phosphorylation of p70S6K and 4E-BP1 induced by arginine or leucine in rat intestinal cells.[20] Because *in vitro* observations of the regulation of the mTORC1 pathway are not always physiologically relevant, it is still unclear whether and how glutamine regulates the mTORC1 pathway in the intestine *in vivo*.

In conclusion, the present study indicates that the mTORC1 pathway plays a critical role in the *in vivo* maintenance of the intestinal mucosa by the oral intake of amino acids. These findings provide a basis for a new approach to preventing intestinal mucosal atrophy and the resulting clinical problems. The precise molecular mechanism by which the overall amino acid concentration or the concentrations of individual amino acids are sensed by and mediated through mTORC1 in the intestinal mucosa remains to be determined.

Acknowledgements

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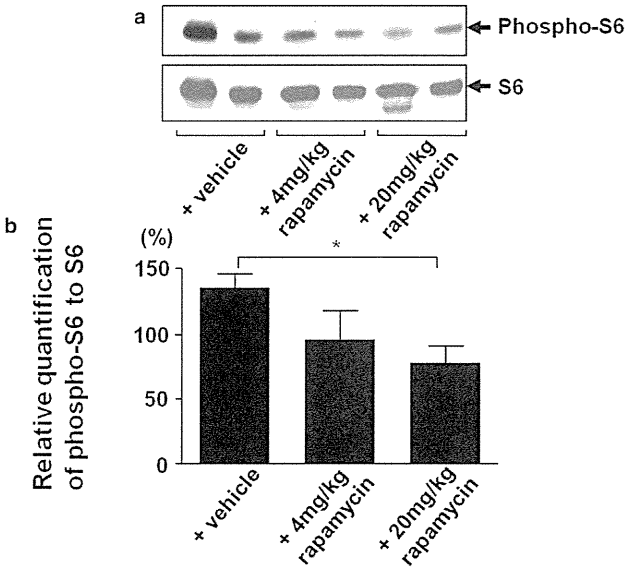

Figure 6 Suppression of the S6 ribosomal protein phosphorylation in the jejunal mucosa by rapamycin. (a) Intestinal mucosa was prepared from mice treated as described in Figure 5, and the same amount of protein extract was analyzed by immunoblotting with antibodies to phospho-S6 ribosomal protein (Ser235/236) or the S6 ribosomal protein. (b) Relative quantification of phospho-S6 (Ser235/236) to S6. The immunoblots were scanned and the optical density of phospho-S6 ribosomal protein (Ser235/236) blot was divided by the optical density of the corresponding S6 ribosomal protein. Data are the means ± SD of values from 6–11 (b) animals from each group. $*P < 0.05$.

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Short Report: Pathophysiology

Development of fulminant Type 1 diabetes with thrombocytopenia after influenza vaccination: a case report

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Abstract

Background Fulminant Type 1 diabetes was originally reported as idiopathic Type 1 diabetes. Involvement of viral infections in the pathogenesis of fulminant T1D has been suggested, but the development of fulminant Type 1 diabetes after influenza vaccination has not been reported.

Case Report We report a case of fulminant Type 1 diabetes with thrombocytopenia following influenza vaccination. A 54-year-old man was admitted to hospital with hyperglycaemia and diabetic ketosis. Seven days before admission, he received a seasonal influenza vaccine for the prevention of influenza infection. On admission, blood glucose was 29 mmol/L and HbA1c 40 mmol/mol (5.9%). Fasting and 2-h C-peptide immunoreactivity were <0.0333 nmol/L and 0.0999 nmol/L, respectively. Anti-GAD and anti-IA-2 antibodies were negative, so no autoimmunity seemed to participate in the etiology. ELISPOT assay also showed no association with T cell-mediated autoimmunity. HLA genotypes were consistent with susceptibility to fulminant Type 1 diabetes. After the abrupt onset of diabetes, he showed mild thrombocytopenia, which has been observed for approximately 5 years after diabetes development.

Conclusion This is the first description of fulminant Type 1 diabetes after influenza vaccination. Our observation raises the possibility that influenza vaccination might trigger this condition via the TLR7 pathway.

Diabet. Med. 29, 88–89 (2012)

Keywords fulminant Type 1 diabetes, influenza, thrombocytopenia, TLR7, vaccination

Introduction

Fulminant Type 1 diabetes was originally reported as idiopathic Type 1 diabetes and accounts for approximately 20% of acute-onset Type 1 diabetes in Japan. This disease is characterized by extremely rapid onset, with hyperglycaemia, near-normal HbA_{1c} and low C-peptide immunoreactivity (CPR) level. In addition, the patients usually show no detectable islet-related autoantibodies, such as islet cell antibody (ICA), insulin autoantibody (IAA), anti-glutamic acid decarboxylase (anti-GAD) antibody or anti-insulinoma associated antigen-2 (anti-IA-2) antibody and elevated pancreatic enzyme levels [1]. Here, we report a case of fulminant Type 1 diabetes with thrombocytopenia following influenza vaccination.

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Case report

A 54-year-old man was admitted to hospital with hyperglycaemia and diabetic ketosis. He had a family history of Type 2 diabetes, whereas he himself had been in good health without a history of hyperglycaemia. Seven days before admission, he received a seasonal influenza vaccine for the prevention of influenza infection. Four days after vaccination, he had general fatigue, thirst and polyuria, without flu-like symptoms. On admission, he had lost 4.5 kg of weight over a period of 2 days and his BMI was 20.2 kg/m^2. Blood glucose was 29 mmol/l and HbA_{1c} 40 mmol/mol (5.9%). In addition,

serum fasting and 2-h C-peptide immunoreactivity were < 0.0333 nmol/l and 0.0999 nmol/l, respectively. Anti-GAD and anti-IA-2 antibodies were negative. Serum amylase was increased to 517 U/l. Taking these factors into consideration, he was diagnosed as having fulminant Type 1 diabetes. Human leukocyte antigen genotypes were DRB1 0405/0901 and DQB1 0303/0401, recently reported to be susceptible to acute-onset and fulminant Type 1 diabetes [2]. Interestingly, after the abrupt onset of diabetes, he showed mild thrombocytopenia (105×10^9 /l) following a transient elevation of platelet count (230×10^9/l), and relative lymphopenia (less than 21% of white blood cells), especially in CD8 T-cells. Thrombocytopenia ($90–130 \times 10^9$/l) with relative lymphopenia has been observed for approximately 5 years after diabetes development. Furthermore, other than for influenza A, he showed no significant elevation of viral antibody titres for influenza B, coxsackie B3 and B4, herpes simplex and human herpes virus 6, even more than 4 weeks later. He had been treated with intensive insulin therapy, whereas he showed neither improvement of insulin secretion nor reduction of daily insulin requirement. To improve glycaemic control, continuous subcutaneous insulin infusion was started recently. To directly evaluate the association with T-cell-mediated autoimmunity, enzyme-linked immunospot (ELISPOT) assay was performed using his peripheral lymphocytes [3]. Neither interferon (IFN)-γ nor interleukin (IL)-4 secretion from T-cells were detected in response to islet-related autoantigens such as GAD65, insulin B9-23 peptide or insulin A1-15 peptide, although some cases of fulminant Type 1 diabetes represented T-cell reactivities [3].

Discussion

Involvement of viral infections in the pathogenesis of fulminant Type 1 diabetes has been suggested, as flu-like symptom existed in 71.7% of the patients just before disease onset [1]. Indeed, association between fulminant Type 1 diabetes and viral infections such as influenza B, coxsackie or herpes viruses has been reported [4]. In this case, however, the patient had neither viral infections nor flu-like symptom before disease development. In contrast, no evidence linking viral vaccines with Type 1 diabetes has been found, although adverse events following influenza vaccination, such as Guillan–Barre syndrome, have been reported [5]. In addition, although relative lymphopenia is reported to be a constant finding in influenza A, but not in acute viral infections, thrombocytopenia with relative lymphopenia has been reported to be extremely rare as an adverse event after influenza vaccination, but not as a viral infection [5,6]. Thrombocytopenia is considered to be mediated by diverse mechanisms such as autoimmunity, but, in this case, platelet-associated immunoglobulin G and *Helicobacter pylori* immunoglobulin G were undetectable. Recent reports suggested that immune responses to influenza virus infection and vaccination were governed through the single-stranded RNA sensor TLR7, currently detected in the pancreas of patients with recent-onset fulminant Type 1 diabetes [7,8]. Taking these reports together, it appears that TLR7 may play a key role in both antiviral defence and disease induction, suggesting that influenza vaccination might trigger abrupt diabetes progression via the TLR7 pathway.

In conclusion, this is the first description of fulminant Type 1 diabetes after influenza vaccination. Our observation suggests the possibility that influenza vaccination might trigger fulminant Type 1 diabetes.

Competing interests

Nothing to declare.

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COMMISSION REPORT

Guidelines for non-medical care providers to manage the first steps of emergency triage of elderly evacuees

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On 11 March 2011, a strong earthquake occurred off of Japan's Pacific coast and hit northeastern Japan. The earthquake was followed by huge tsunamis, which destroyed many coastal cities. As a result, the Study Group on Guidelines for the First Steps and Emergency Triage to Manage Elderly Evacuees quickly established guidelines enabling non-medical care providers (e.g. volunteer, helpers, and family members taking care of elderly relatives), public health nurses, or certified social workers to rapidly detect illnesses in elderly evacuees, and 20 000 booklets were distributed to care providers in Iwate, Miyagi, and Fukushima prefectures. The aim of this publication is to reduce susceptibility to disaster-related illnesses (i.e. infectious diseases, exacerbation of underlying illnesses, and mental stress) and deaths in elderly evacuees. **Geriatr Gerontol Int 2011; 11: 383–394.**

Keywords: earthquake, elderly evacuee, emergency triage, guidelines, non-medical care provider.

Background

Japanese people have already experienced a variety of natural disasters including earthquakes,[1] typhoons,[2] tsunamis,[3] and others. It is very important to manage the medical care of elderly evacuees in the wake of disasters because: (i) elderly subjects (especially those needing to live in shelters) may suffer excessive mental and/or physical stress under the altered environment; and (ii) it is difficult to maintain medical management of chronic illnesses (e.g. hypertension, diabetes mellitus, cerebrovascular or cardiac disease) when care has already been started at local medical institutions. It was reported that acute risk factors possibly triggered cardiovascular events in hypertensive elderly patients after the Hanshin-Awaji earthquake.[4] Increased incidence of transient left ventricular apical ballooning (takotsubo cardiomyopathy) was also described after the Mid Niigata Prefecture Earthquake of 2004.[5]

In April 2010, the Study Group on "Guidelines for the First Steps and Emergency Triage to Manage Elderly

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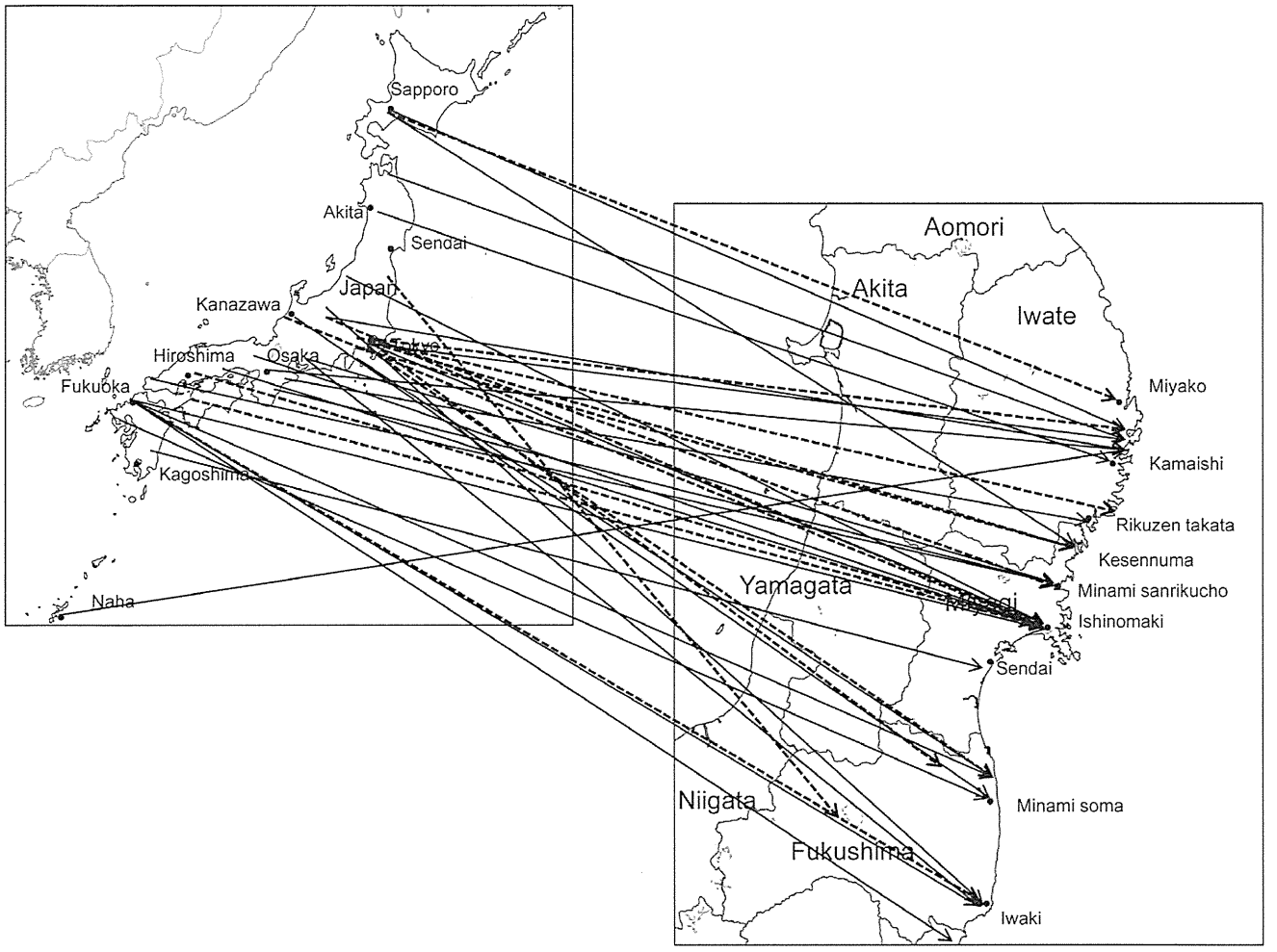

Figure 1 One week after the 2011 Tohoku earthquake, 20 000 booklets for non-medical care providers were distributed by members of the Japan Geriatrics Society (dotted lines) and Japan Medical Association Team (straight lines), to evacuation centers located in Iwate, Miyagi, and Fukushima prefectures.

Evacuees" was formed, with funding from Japan's Ministry of Health, Labour and Welfare, to conduct comprehensive research on aging and health. The study group aimed to complete and revise the guidelines based on external reviews by expert medical doctors by March 2012.

By collaborating with the Japan Geriatrics Society after the 2011 earthquake off the Pacific coast of Tohoku, we have quickly published two tentative guidelines to manage elderly evacuees: one for medical care providers and another for non-medical care providers (NMCP), including volunteer, helpers, and family members who are taking care of the elderly, public health nurses (PHN), or certified social workers (CSW). A total of 20 000 guideline booklets have been distributed by members of the Japan Geriatrics Society and the Japan Medical Association Team to NMCP, PHN, or CSW working in Iwate, Miyagi, and Fukushima prefectures (Fig. 1). The Japan Medical Association Team's mission is to provide medical assistance at hospitals or clinics in disaster-affected areas and to provide ongoing medical treatment that was started before the disaster.[6]

Preface

The guidelines for NMCP, PHN, and CSW have three chapters: (i) Features and prevention of critical diseases in elderly in evacuation areas; (ii) Signs of acute diseases in elderly; and (iii) Symptoms of anxiety in elderly in shelters. Ideally, NMCP, PHN, or CSW will use the booklets to rapidly detect illnesses in the elderly in shelters or homes. NMCP, PHN, or CSW should immediately inform attending medical staff when those with the signs or symptoms are detected.

Guidelines

I. Features and prevention of critical diseases in elderly in evacuation areas

1–1). Heart attack. This condition includes angina pectoris, myocardial infarction, and other illnesses due to myocardial ischemia, a lack of blood flow in arteries.

Signs and symptoms of a heart attack

Location of symptoms	Central chest to left side of chest Apart from chest discomfort, anginal pain in the upper central abdomen, back, neck, jaw, or shoulders
Detailed symptoms	Worsening ("crescendo") chest pain, specifically crushing, burning, or choking sensation Onset of severe oppression or worsening oppression
Duration of symptoms	Infrequent or lasting less than 10 min Lasting more than 15 min, suggesting unstable condition

Note: Caution is needed because silent or mild symptoms frequently occur in the elderly, especially in those with diabetes. In addition, elderly people sometimes present with atypical symptoms, including breathlessness, nausea, discomfort in the upper central abdomen, or burping.

Measures to prevent heart attack in shelters

- NMCP, PHN, or CSW should be aware of elderly who normally take medication for cardiac disease and/or hypertension.
- NMCP, PHN, or CSW should check on the elderly.
- NMCP, PHN, or CSW should ensure that the elderly drink plenty of fluid, including water, to prevent dehydration. They should also advise that the elderly consume a low-salt diet and not smoke.
- If the elderly have any of the above symptoms, medical staff should be alerted.

Tips to treat cardiopulmonary arrest in shelters

- NMCP, PHN, or CSW should perform CPR, pushing the central chest strongly and quickly (100 times per minute) and alert medical staff immediately.

1–2). Hypertension. Awareness of blood pressure (BP) and its variability in the elderly is necessary because they may have excessive mental and/or physical stress, especially if in an emergency evacuation area or first-aid station, relative to their day-to-day lives before the disaster.

Measures to deal with elderly receiving antihypertensive drugs

- First, elderly people who are usually prescribed antihypertensive drugs should be reported to medical staff. NMCP, PHN, or CSW should check on the elderly.
- Elderly people who have been diagnosed as hypertensive should also be checked by medical staff, NMCP, PHN, or CSW.
- BP should be measured frequently. If possible, it is better to measure it daily using an automatic BP machine. In high-risk patients, it is recommended that BP be measured in both the morning and evening.
- If the elderly person's medication is not known because the prescription record is lost, a doctor or medical staff should be consulted.
- If an elderly person has a headache, palpitations, chest symptoms, and/or flushing, BP should be measured immediately and medical staff consulted.
- No smoking and a low-salt diet are also recommended. Endeavors must be made to ensure the elderly maintain physical activity (e.g. any exercise for at least 30 minutes a day).

2. Stroke/cerebrovascular disease (CVD)

Cerebrovascular accidents occur suddenly due to a disturbance in the blood supply to the brain and lead to a loss of cerebral function.

Signs and symptoms of stroke/CVD

If elderly people have any of the following symptoms, it is possible that they may have suffered a stroke/CVD. Consult medical staff immediately, because these situations may become medical emergencies.

- Symptoms starting suddenly and lasting from a few seconds to minutes
- Headache (mild to severe)
- Vertigo and/or dizziness (with nausea/vomiting on occasion)
- Disturbance of consciousness (snoring-like breathing, semiconscious state/coma)
- Motor disturbance including hemiparesis/hemiplegia/numbness, exhaustion, muscle weakness of the face (central facial palsy), drooling from one corner of the mouth, eyelid drooping (ptosis)
- Aphasia (difficulty with verbal expression, auditory comprehension)
- Sensory or vibratory disturbance (on one side)
- Visual field defect/hemianopia, double vision/polyopia
- Loss of balance when sitting, standing, or walking; loss of coordination.

Measures to prevent stroke/CVD in shelters

- First, medical staff and people around should be aware of elderly people who usually take medication for atherosclerotic diseases and/or lifestyle-related diseases (e.g. hypertension, diabetes, dyslipidemia, and cardiac diseases including atrial fibrillation).
- Also, people around should check on the elderly.