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IV. 研究成果の刊行物・別冊

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ORIGINAL ARTICLE

Efficacy and safety of long-term, continuous subcutaneous octreotide infusion for patients with different subtypes of K_{ATP}-channel hyperinsulinism

Tohru Yorifuji*, Rie Kawakita*, Yuki Hosokawa*, Rika Fujimaru*, Kousaku Matsubara†, Katsuya Aizu‡, Shigeru Suzuki§, Hironori Nagasaka¶, Hironori Nishibori** and Michiya Masue††

*Department of Pediatric Endocrinology and Metabolism, Children's Medical Center, Osaka City General Hospital, Osaka, †Department of Pediatrics, Nishi-Kobe Medical Center, Kobe, ‡Division of Endocrinology and Metabolism, Saitama Children's Medical Center, Saitama, §Department of Pediatrics, Asahikawa Medical University, Asahikawa, ¶Department of Pediatrics, Takarazuka City Hospital, Takarazuka, **Department of Radiology and ††Department of Pediatrics, Kizawa Memorial Hospital, Minokamo, Japan.

Summary

Objective To evaluate the efficacy of long-term, continuous, subcutaneous octreotide infusion for congenital hyperinsulinism caused by mutations in the K_{ATP}-channel genes, *KCNJ11* and *ABCC8*.

Patients Fifteen Japanese patients with diazoxide-unresponsive, K_{ATP}-channel hyperinsulinism.

Methods Molecular diagnoses were made by sequencing and multiple ligation-dependent probe amplification analysis. In patients with paternally inherited, monoallelic mutations, 18F-DOPA PET scans were performed to determine the location of the lesion. The patients were treated with continuous, subcutaneous octreotide infusion at a dosage of up to 25 μg/kg/day, using an insulin pump to maintain blood glucose levels higher than 3·33 mmol/l. Additional treatments (IV glucose, glucagon or enteral feeding) were administered as needed. The efficacy of the treatment was assessed in patients who received octreotide for 4 months to 5·9 years.

Results Three patients had biallelic mutations, and 12 had monoallelic, paternally inherited mutations. Four patients with monoallelic mutations showed diffuse 18F-DOPA uptake, whereas seven patients showed focal uptake. Octreotide was effective in all the patients. The patients with biallelic mutations required a higher dosage (17–25 μg/kg/day), and two patients required additional treatments. By contrast, the patients with monoallelic mutations required a lower dosage (0·5–21 μg/kg/day) irrespective of the PET results and mostly without additional treatments. Treatment was discontinued in three patients at 2·5, 3·3 and 5·9 years of age, without psychomotor delay. Except for growth deceleration at a higher dosage, no significant adverse effects were noted.

Conclusions Long-term, continuous, subcutaneous octreotide infusion is a feasible alternative to surgery especially for patients with monoallelic K_{ATP}-channel mutations.

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Introduction

Congenital hyperinsulinism (CHI) is the most common cause of persistent hypoglycaemia in the neonatal and infantile periods with an incidence of approximately 1 in 40 000 births in Japan.

Although a variety of causative genes have been identified for this disorder,[1] when restricted to diazoxide-unresponsive cases, defects in the ATP-sensitive potassium channel (K_{ATP}-channel) are by far the most commonly associated alterations, accounting for 92% in our series of 48 Japanese cases of diazoxide-unresponsive persistent CHI (our unpublished results).

K_{ATP} CHI is caused by loss-of-function mutations in 1 of 2 genes, *ABCC8* or *KCNJ11*, encoding the two subunits of the pancreatic K_{ATP}-channel, SUR1 and Kir6·2, respectively. Two major histological forms of K_{ATP} CHI are known: the diffuse and focal forms. In the diffuse form, insulin-oversecreting abnormal β cells are distributed throughout the pancreas, whereas in the focal form, abnormal β cells occupy a restricted area in the pancreas. The diffuse forms are caused either by biallelic, recessively inherited K_{ATP}-channel mutations, or by monoallelic, dominantly inherited mutations.[2] On the contrary, the focal forms are found in individuals with a paternally inherited, monoallelic K_{ATP}-channel mutation.[1,3–5] Subsequent somatic loss of the maternal allele caused by paternal isodisomy leads to a loss of the activities of the K_{ATP}-channel and the

Correspondence: Tohru Yorifuji, Department of Pediatric Endocrinology and Metabolism, Children's Medical Center, Osaka City General Hospital. 2-13-22 Miyakojima-Hondori, Miyakojima, Osaka 534-0021, Japan.
Tel.: +81 6 6929 1221; Fax: +81 6 6929 1090;
E-mail: t-yorifuji@hospital.city.osaka.jp

adjacent tumour suppressors (*H19* and *CDKN1C*) normally expressed by the maternal allele. These cells gain a growth advantage, eventually forming a focal lesion of insulin-overproducing β cells.[1,3–5]

Traditionally, patients with CHI showing a poor response to medical treatment have been treated by subtotal pancreatectomy, removing > 95% of the pancreas to prevent serious neurological sequelae of hypoglycaemia.[6] The results of the procedure, however, were rarely satisfactory. Some were left with residual hypoglycaemia, and even when hypoglycaemia was controlled, most of the patients developed diabetes mellitus postoperatively.[7]

This situation has changed recently. When a focal lesion is identified preoperatively, the patient can be cured by partial resection of the pancreas, without postoperative complications.[1,8] With the recent development of diagnostic modalities for the identification and localisation of focal lesions, surgical treatment by a multidisciplinary approach has become the mainstay of treatment for focal diazoxide-unresponsive CHI.[1]

Even today, however, patients with the diffuse form of K_{ATP} CHI often require subtotal pancreatectomy with frequent postoperative complications.[9] In addition, when a focal lesion is identified, surgical resection is not always easy if the lesion is in the head of the pancreas and potentially adjacent to the main pancreatic duct or the common bile duct.[10] Total removal of the head of the pancreas and drainage of the distal pancreas into the Roux-en-Y jejunal loop has been advocated for such cases.[10,11] The procedure, however, can be difficult for paediatric surgeons who do not routinely perform surgery for the correction of this rare disorder, and the frequency of long-term postoperative complication is not known. Because approximately two-thirds of the focal lesions arise in the head or the uncus of the pancreas (our unpublished results in Japan), this problem occurs frequently.

Octreotide, a synthetic somatostatin analogue, is currently used for treatment of CHI.[12–14] Although long-term treatment with octreotide has been reported,[15–18] the treatment regimen differs from centre to centre, and few reports have correlated its efficacy and safety with subtypes of hyperinsulinism. In this study, we evaluated the efficacy and safety of long-term octreotide treatment in Japanese patients with genetically proven K_{ATP} CHI. The amount of octreotide to control hypoglycaemia was then correlated with the genotype and 18F-DOPA PET findings.

Subjects and methods

Subjects

Fifteen Japanese patients with diazoxide-unresponsive K_{ATP} CHI who were treated with continuous subcutaneous octreotide infusion for 4 months to 5·9 years were included in the study. At presentation, all patients showed blood glucose below 2·5 mmol/l (45·05 mg/dl) and insulin level greater than 25 pmol/l (3·6 μU/ml). The patients first underwent molecular diagnosis at Osaka City General Hospital while blood glucose levels were maintained by continuous glucose infusion. Then, the patients who were suspected with a possible focal K_{ATP} CHI, because a paternally inherited monoallelic mutation was found in either *ABCC8* or *KCNJ11*, were further assessed by 18F-DOPA PET scan at Kizawa Memorial Hospital. The patients showing diffuse uptake or focal uptake in the head of the pancreas, which could be difficult to enucleate, were preferentially enrolled into the long-term octreotide treatment.

Methods

Mutational analysis. Mutational analysis of the K_{ATP}-channel genes, *KCNJ11* and *ABCC8*, was performed as described previously.[19] All exons and exon–intron boundaries were amplified from genomic DNA and directly sequenced. Deletion mutations that might not have been detected by the PCR-sequencing strategy were analysed by multiple ligation-dependent probe amplification (MLPA) of all 39 exons of the *ABCC8* gene. The analyses were performed using SALSA MLPA kit P117 (MRC Holland, Amsterdam) as recommended by the manufacturer.

18F-DOPA PET. 18F-DOPA PET studies were performed at the PET facility of Kizawa Memorial Hospital, as described previously.[20] The scan results were fused with those of a CT scan performed at the same time to localise the focal lesion more accurately.

Octreotide treatment. The patients were treated with continuous, subcutaneous octreotide infusion using an insulin pump to maintain blood glucose levels higher than 3·33 mmol/l (60 mg/dl).[21] Starting at a lower dose, the amount of octreotide was titrated up to 25 μg/kg/day to minimize the amount of IV glucose. Blood glucose was measured at least eight times a day at the initiation of the treatment by using a portable glucometer, and 1–4 times a day at home after the patient was discharged. The age of the patients at the initiation of octreotide was 11 day to 12 month. In four patients (patients 4, 11, 13, 14), octreotide was first initiated by multiple daily injections to confirm its effectiveness and then switched to continuous infusion within 4 weeks. Other treatments immediately before and after the initiation of octreotide are shown in Table 1. After the initial stabilization of blood glucose and after the age of 4 months, most infants who did not undergo pancreatectomy were fed cornstarch up to 2 g/kg at bedtime to help reduce the required dosage of octreotide. Additional treatments (IV glucose, glucagon or enteral feeding) were administered as needed to maintain normoglycaemia. The study was conducted in accordance with the Declaration of Helsinki (British Medical Journal, 1964, ii, 177) after obtaining informed consent from the guardians, and the protocols for molecular diagnosis, 18F-DOPA PET and octreotide treatment were approved by the institutional review boards of each participating hospital.

Clinical and laboratory data collection. Clinical and laboratory data, which were collected from the medical record of each patient, included gestational age; birth weight; blood glucose and insulin at diagnosis; details of octreotide treatment and any

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Table 1. Characteristics of the patients enrolled in the study

No	Gene	Mutation (amino acid change)	Localisation by 18F-DOPA PET	Gender	Age at onset	Octreotide			Changes in clinical status before and after octreotide infusion		Current clinical status
						Initial dose (μg/kg/day)	Age at start	Duration of octreotide	Before	After	
1	ABCC8	c.1773delC (p.F591Ffs*14) c.2992C>T (p.R998*)	ND	M	0 day	25	11 day	2·2 years	IVG (4), GCG,	IVG (1·1), GCG	90% pancreatectomy followed by octreotide
2	ABCC8	c.2506C>T (p.R836*) c.4575_4587del13 (p.T1525Tfs*15)	ND	M	2 days	17	27 days	2·7 years	IVG (8·3)	EF	Continued on octreotide
3	ABCC8	c.2506C>T (p.R836*) c.4412-13G>A (p.?)	ND	F	0 day	17	5 months	5·9 years	IVG (16)	None	Continued on octreotide
4	ABCC8	c.2992C>T (p.R998*)	body	M	0 days	4	28 days	4 months	IVG (15), DZX	None	Cured by partial resection (0 years 4 months)
5	ABCC8	c.2992C>T (p.R998*)	body	M	5 months	0·5	12 months	1·5 years	IVG (3)	None	Spontaneous remission (2 years 6 months)
6	ABCC8	c.2506C>T (p.R836*)	uncus	F	1 months	10·95	10 months	1·5 years	IVG (6·86)	None	Cured by partial resection (1 years 6 months)
7	ABCC8	c.2506C>T (p.R836*)	diffuse	M	0 days	12·5	17 days	3·3 years	IVG (8), GCG	None	Spontaneous remission (3 years 3 months)
8	ABCC8	c.62 insG (p.V21Gfs*67)	diffuse	F	2 months	3·2	4 months	5·6 years	IVG (9·6)	None	Spontaneous remission (5 years 11 months)
9	ABCC8	c.2506C>T (p.R836*)	diffuse	F	7 months	2·3	8 months	4·6 years	IVG (5·95)	None	Continued on octreotide
10	ABCC8	c.4307G>A (p.R1436Q)	diffuse	M	7 months	5	7 months	5 months	IVG (3·1)	None	Continued on octreotide
11	ABCC8	c.4608 + 1G>C (p.?)	head	F	1 days	12	21 days	7 months	IVG (13)	None	Partial resection followed by octreotide
12	KCNJ11	c.350_352del TCT (p.F117_S118delinsS)	head	F	6 months	3	8 months	1·8 years	IVG (2·4)	None	Continued on octreotide
13	ABCC8	c.68delA (p.N23Tfs*55)	head	M	6 months	9	7 months	4 months	IVG (5)	None	Continued on octreotide
14	ABCC8	c.62T>A (p.V21D)	head	M	1 days	6·4	2 months	5 months	IVG (4)	None	Continued on octreotide
15	KCNJ11	c.405_406insG (p.L136Afs*136)	ND	F	5 m	21	8 months	1·6 years	IVG (6·5)	None	Continued on octreotide

*GCG, glucagon; IVG, intravenous glucose (glucose infusion rate, mg/kg/min); EF, enteral feeding, DZX, diazoxide.
The demographic features of each patient are summarised together with the results of the molecular analyses, 18F-DOPA PET and other parameters related to the octreotide treatment. ND, not done.

additional treatment required to achieve euglycaemia; laboratory results including complete blood counts, blood chemicals (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, total protein, albumin, urea nitrogen, creatinine, total cholesterol, triglyceride), electrolytes, thyroid function tests and abdominal ultrasound; reports of short-term adverse effects such as abdominal symptoms; and long-term neurological and growth outcomes when applicable.

Results

Table 1 provides a summary of the demographic features of the patients, as well as the genotype, 18F-DOPA PET findings, details of the octreotide treatment and clinical outcomes. All identified mutations were point mutations, and no exonic deletions were found by MLPA. As reported previously,[19] monoallelic paternal K_{ATP}-channel mutations are predominant in Japan and the molecular breakdown also reflected this trend. Three patients had biallelic mutations of the K_{ATP}-channel genes, and the remaining 12 had monoallelic paternal mutations suggestive of the possible focal form. 18F-DOPA PET scans were performed in 11 of the patients with monoallelic mutations. Four of them showed apparently diffuse uptake, and the remaining seven showed focal uptake: five in the head or the uncus and two in the body of the pancreas.

The octreotide treatment was effective, at least partially, in all the patients (Table 1). For the patients with biallelic mutations (patients 1–3), octreotide was less effective requiring a higher dosage than for the patients with monoallelic mutations (17–25 μg/kg/day); mean, 19·7 μg/kg/day; $P = 0{\cdot}02$ by the Welch *t*-test), and two of them required additional treatment with hypertonic intravenous glucose infusion (patient 1) or enteral feeding (patient 2) to maintain normoglycaemia. As IV glucose could not be completely stopped, patient 1 underwent 90% pancreatectomy at the age of 4 months. After surgery, the patient continued to experience hypoglycaemia, which could be controlled with a lower octreotide dosage (12 μg/kg/day) without additional IV glucose. These patients with biallelic mutations were still being treated with octreotide at the time of the study at 2, 2 and 6 years of age, respectively. However, the required octreotide dosage was decreased to 6·5, 15 and 7·6 μg/kg/day, respectively.

By contrast, the patients with monoallelic mutations required a lower dosage (0·5–21 μg/kg/day; mean, 6·25 μg/kg/day), mostly without additional treatments. There were no significant differences in dosage requirements between the different PET signal patterns, with a dosage of 2·3–12·5 μg/kg/day (mean, 5·8 μg/kg/day) administered to the patients with diffuse uptake and paternal monoallelic mutations as compared with the 0·5–21 μg/kg/day (mean, 6·5 μg/kg/day) administered to the patients with focal uptake ($P = 0{\cdot}78$ by the Welch *t*-test).

Three patients with a focal 18F-DOPA uptake (patients 4, 6, 11) underwent partial pancreatectomy. Two of them (patient 4, 6) were cured, and octreotide treatment was discontinued at the time of the surgery. One (patient 11) remained hypoglycaemic after surgery due to incomplete resection of the focal lesion, and octreotide treatment was therefore continued at the same dosage.

Nine other patients with monoallelic mutations (four diffuse, four focal and one unknown uptake of 18F-DOPA) chose to continue the octreotide treatment without surgery. Three of them (patients 5, 7, 8) actually achieved remission at the age of 2·5, 3·3 and 5·9 years, respectively, requiring no additional treatment. These patients were subjected to continuous glucose monitoring for 3 days without any treatment, and no episodes of hypoglycaemia were reported. The remaining six patients with monoallelic mutations were still receiving octreotide. The dosage of octreotide, however, was gradually decreasing with age. In patient 9, after 4·6 years of treatment, the dosage could be decreased to 0·6 μg/kg/day as she was prepared for treatment discontinuation.

None of the 15 patients showed obvious psychomotor retardation. Patients 2, 3, 7, 8, 9, who underwent extended octreotide treatment, showed normal results in the formal assessment of the developmental quotients at 112, 82, 94, 102 and 107 (normal range >70), respectively (Table 1).

With regard to the route of administration, continuous infusion of octreotide seemed to be superior to multiple daily injections. Of the four patients who were converted to continuous subcutaneous infusion from multiple daily injections, two were able to tolerate a decrease in the required octreotide dosage from 18 to 9 μg/kg/day (patient 13) and from 8 to 6·4 μg/kg/day (patient 14). In the remaining two patients (patients 4 and 11), there were no changes in the octreotide dosage. However, in patient 4, continuous nocturnal nasogastric tube feeding could be discontinued after conversion to continuous infusion at the same dosage as that used for multiple injections.

Overall, except for the transient gastrointestinal symptoms (poor appetite, constipation, or change in stool colour) observed in three patients (patients 11, 13, 14), the treatment was well tolerated. Laboratory test results revealed no significant changes in the blood count, blood chemicals, serum electrolyte concentration and thyroid function during the course of the treatment, and gallstones did not develop in any of the patients. However, at the higher dosages (>17 μg/kg/day), growth deceleration was observed in two patients (patients 2 and 3) with biallelic mutations (Fig. 1). The deceleration appeared to be caused by the suppression of growth hormone secretion due to the octreotide treatment. In patient 3, serum IGF1 measurements were 16·3 nmol/l at 2·0 years of age, 16·5 nmol/l at 3·0 years and 11·4 nmol/l at 4·0 years. Growth hormone provocation tests performed at the age of 4·0 years showed reduced peak growth hormone values of 1·43 μg/l by the levodopa loading test and 3·33 μg/l by the clonidine loading tests (cut-off, 6 μg/l). Because the growth deceleration was more significant in patient 3, the octreotide dosage was gradually decreased from 17 μg/kg/day to 8 μg/kg/day during the age of 4 years, which resulted in the recovery of the growth rate accompanied by an increase in the serum IGF1 to 24·5 nmol/l at the age of 6 years, indicating that the suppression of growth and growth hormone secretion were dose dependent. Growth deceleration was negligible in the

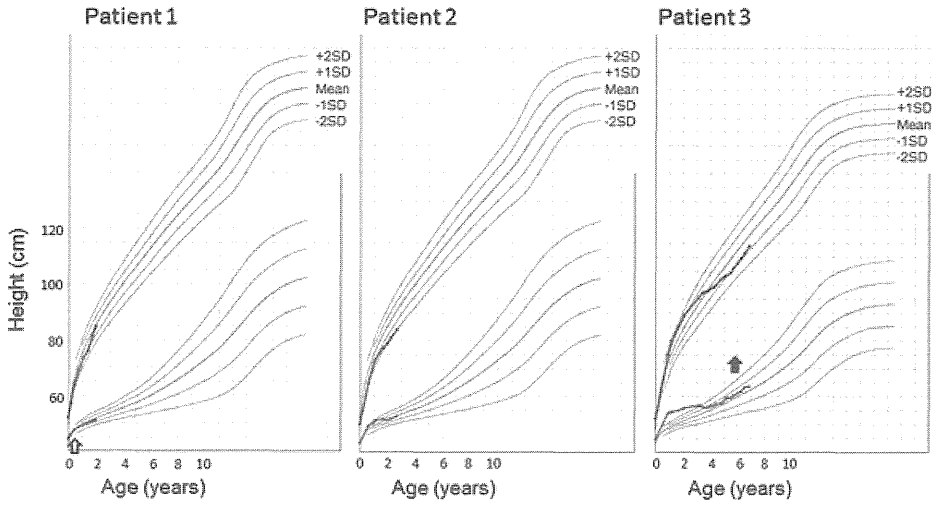

Fig. 1 Growth curve of patients with biallelic K_{ATP}-channel mutations treated by long-term, subcutaneous octreotide infusion (patients 1–3). A tendency towards growth deceleration was observed in patients 2 and 3 after 2 years of age, loss of approximately 1 and 2 SD of height, respectively. Growth deceleration was not observed in patient 1, who underwent 90% pancreatectomy and continued on octreotide at a lower dosage. The open arrow in the left panel shows when patient 1 underwent surgery, and the closed arrow in the right panel shows when the octreotide dosage was reduced from 17 to 8 μg/kg/day in patient 3, indicating the dose dependency of growth deceleration.

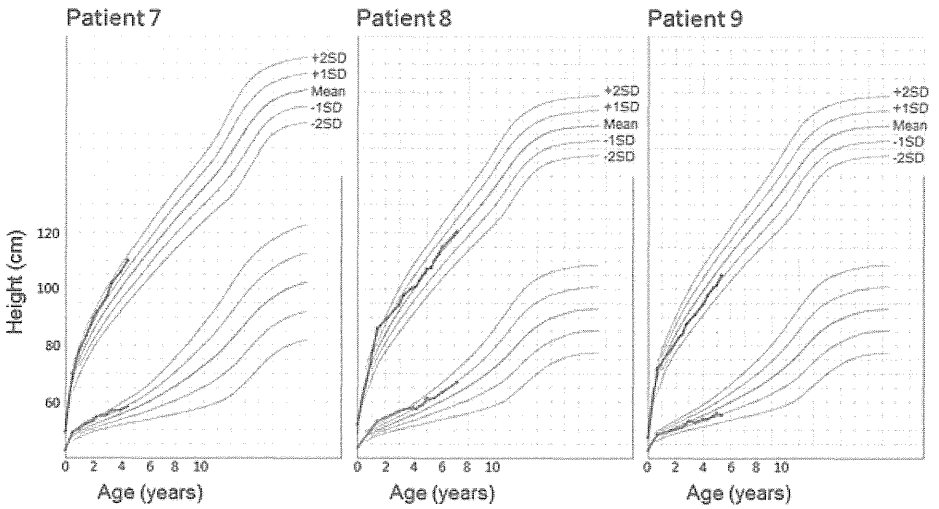

Fig. 2 Growth curve of the patients with monoallelic K_{ATP}-channel mutations treated with long-term, subcutaneous octreotide infusion. No obvious growth deceleration was observed.

patients with monoallelic mutations who were treated with a lower octreotide dosage (Fig. 2).

Discussion

The results of our study indicate that primary long-term, continuous, subcutaneous octreotide infusion with the purpose of achieving spontaneous remission could be an alternative treatment, especially in patients with monoallelic paternal mutations and in those showing diffuse or focal uptake in the head or uncus of the pancreas on 18F-DOPA PET scan. Without additional treatment such as continuous IV glucose infusion or enteral feeding, the burden of the long-term treatment was similar to that of the insulin pump therapy for infantile diabetes, which was acceptable at least to the guardians included in our study.

For patients with biallelic mutations, the situation is somewhat different. The required octreotide dosage is large, which may lead to growth deceleration after infancy. The treatment periods tend to be longer, and very often, additional treatments are required, making the long-term management of these patients more difficult. One possible strategy for such patients could be elective partial resection of the pancreas for mass reduction, as performed for patient 1, and postoperative continuous octreotide treatment until spontaneous remission is achieved. The assessment of a larger number of cases is necessary to establish the best strategy for these patients.

In our series, the octreotide dosage required to achieve euglycaemia did not differ between the patients with diffuse and focal uptakes on the 18F-DOPA PET scan, as long as they had paternally inherited monoallelic mutations. Most monoallelic K_{ATP}-channel mutations are paternal,[19,22] suggesting the presence of a focal lesion. However, PET scan results show that a proportion of these patients actually present with a diffuse uptake.[19,22] Whether this represents the presence of an undetectable second mutation or unusually scattered focal lesions remains unknown. From a statistical standpoint, selectively missing the maternal mutation is highly unlikely, supporting the latter hypothesis. Our results with regard to the therapeutic response support the hypothesis that diffuse K_{ATP} CHI with monoallelic mutations is more similar to focal K_{ATP} CHI than to recessively inherited K_{ATP} CHI with only a limited number of abnormal β cells scattering widely in the pancreas.

Transient gastrointestinal problems and growth deceleration at higher dose of octreotide were the only adverse events encountered in this study. With regard to the height outcome of the patients, contradictory results have been published previously.[15,17,23] If the observed deceleration in height velocity is caused by suppression of growth hormone secretion, the patient should catch up in growth, at least to some extent, once the octreotide therapy could be stopped before puberty. The discrepancy of our results and those of previously reported height outcome might reflect this difference in the timing of observation. As surgically treated patients were also reported to lose height SDS for unknown reasons,[23] long-term follow-up of a larger cohort is necessary to assess the height outcome according to different treatment modalities.

Although no serious adverse events were noticed during the study period, this could be only due to the small number of the patients enrolled in this study. Presently, the most serious known side effect of octreotide treatment for congenital hyperinsulinism is the development of necrotising enterocolitis.[24] This association could develop in full-term infants and should be given full attention during the octreotide treatment.

As the treatment is not entirely free from serious side effects, the dosage of octreotide should be minimized. In our study, it appeared that, by changing to continuous infusion, the dosage of octreotide could be reduced compared with multiple injections. The serum half-life of octreotide following single subcutaneous injection is known to be 1·5–2 h. Assuming that we need certain serum concentration of octreotide to keep euglycaemia and we inject octreotide three times a day, theoretically, we need much higher dose of octreotide to achieve the same glycaemic goal. This needs to be tested systematically in a larger number of patients, and, in this regard, longer-acting somatostatin analogue such as lanreotide or long-acting release octreotide could also be useful alternatives to continuous octreotide infusion.[25,26]

As the focal form of K_{ATP} CHI is known to be caused by paternal uniparental disomy which, if occurred at the earlier stage of development, leads to Beckwith-Wiedemann syndrome, a theoretical concern common to the long-term treatment of focal K_{ATP} CHI is the possibility of future development of β-cell neoplasm. The patients, therefore, should be followed up for a long time even if they reach spontaneous remission.

In summary, we believe that long-term, continuous, subcutaneous octreotide infusion is a useful alternative to surgery especially for patients with monoallelic K_{ATP}-channel mutations. Because the disorder is relatively rare, the systematic accumulation of a larger number of cases is necessary to establish an optimal treatment regimen as an evidence-based therapeutic strategy.

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