Case report

Nicotinic cholinergic symptoms after consecutive tea drink consumption: Clinical findings of electrocardiography, auditory brainstem response, and infrared pupillography, and acetamiprid residual analysis

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Abstract

In 2005–2007, we studied more than 500 patients who complained of nicotinic symptoms after oral intake of tea drinks (more than 500 ml/day), fruits, or vegetable juice, with high thiocyanate ion residue in the urine. The symptoms of these patients resembled those with nicotine intoxication. Acetamiprid, a cyano-containing neonicotinoid pesticide that acts as nAChR agonist, has rather high food residual threshold in Japan, i.e. 50 ppm for dried tea leaves, 5ppm for apples. In this report we examined the infrared pupillography, auditory brainstem response (ABR), and electrocardiography in three patients with nicotinic symptoms such as headache, nausea, chest pains, dyspnea, finger tremor, muscle pain, sleepiness, memory disturbance and verbal disorders, after consecutive green tea intake. In addition, we analyzed acetamiprid in several kinds of fruits, tea leaves, and tea beverages by HPLC/UV. In the results, we found both sympathotonic and parasympathotonic status of pupil function in three cases and short I-V peak intervals in ABR. Case 1 had severe tachycardia (143 bpm), Case 2 had ST elevation and Case 3 had QT prolongation in electrocardiogram. All patients recovered by detoxication therapy with prohibition of tea drinks and fruits. Acetamiprid was detected from an apple core 4.88+/-0.11 ppm, two kinds of tea leaves made in China 19.88+/-2.07 ppm, 10.72+/-0.00 ppm, and a tea beverage 2.49+/-0.34 ppm (mean+/-SD). The present three cases may give us important clinical suggestion about the sub-acute or chronic toxicity of neonicotinoid, which is used as a major pesticide all over the world.

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《**Key words**》 Acetamiprid, neonicotinoid, pupil, tea, electrocardiogram

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Ⅰ. Introduction

Recently, many reports suggest that a high intake of vegetables, fruits and tea drink are effective for health promotion, improving metabolic syndrome, preventing cancers and reducing body weight. These foods contain essential nutrients, such as vitamins, minerals, dietary fiber, and antioxidants, which are lacking in the typical modern diet; however, it is not always true that foods containing optimal ingredients are useful as a whole.

Taira and Aoyama reported that at least 500 patients, who took relatively higher doses of fresh fruits and green tea for consecutive days, visited A-clinic with complaint of physical disorder, at the meeting of the Japanese Society of Clinical Ecology in July 2007. Their main complaints were headache, vomiting, chest pain, palpitation, chest discomfort, constipation, or diarrhea. These symptoms were occasionally accompanied with muscle weakness and impaired short-term memory, and abnormal activities (hyperactivity and excitability) in children. Their electrocardiogram showed sinus tachycardia that persisted for several days to a week, and/or sinus bradycardia that persisted for several weeks characteristically, and frequently was accompanied with wandering pacemaker, ST change, and QT prolongation. Thiocyanate ion (more than 5 ppm) was detected in nine out of thirteen urine samples randomly taken from the patients, with the maximum level of 15.8 ppm from the urine of a 9-year-old boy. Their symptoms had improved in a short period by the restriction of their intake of domestic vegetables and tea, oral administration of glucronolactone and magnesium supplements, and in some cases intravenous administration of maltose infusion, with a small amount of glutathione added. Their elecrocardiogaphic findings closely resemble those from patients after the aerial spraying of acetamiprid for control of wood boring beetles in pine trees in the summer of 2005, reported by Taira and Aoyama in 2006)[1].

Recently, the use of neonicotinoid pesticide is increasing as an alternative to organophosphorus pesticides, which produce chronic neurotoxicity and teratogenicity in addition to acute toxicity caused by acetyl cholinesterase inhibition. Acetamiprid, ((E)-N^1-[(6-chloro-3-pyridyl) methyl]-N^2-cyano-N^1-methylacetamidine), is a chloropyridinyl neonicotinoid insecticide that possesses cyano-group, was developed in Japan, and is used worldwide in the US, EU, Asia including China, and Oceania.

The authors postulated that the pupillary response of patients who consumed more than 500 mL of tea drink for consecutive days and exhibited nicotine intoxication-like clinical observation and electrocardiography finding, was related to agonism of the nicotinic acetylcholine receptor (nicotinic receptor), which is the effector site of neonicotinoid. We investigated the infrared pupillography and auditory brainstem responses (ABR) in three cases with consecutive consumption of green tea drinks, whose complaints of headache, vomiting, chest pain, palpitation, chest discomfort, constipation or diarrhea, muscle pain, showed abnormal electrocardiograms after the summer of 2006. In addition, to test the hypothesis that the amounts of acetamiprid in food and beverages obtained in the market is below the maximum residual limits, we analyzed residual acetamiprid in commercially available tea leaves, tea beverages, and domestic fruits using high-performance liquid chromatograph with a ultraviolet detector (HPLC/UV).

Ⅱ. Subjects and Methods

Part 1: Case report

We examined infrared pupillography[2] (Electronic Pupilometer, Hamamatsu Photonics, Hamamatsu, Japan, Footnote1) and auditory brainstem responses[3] (ABR, Footnote2) in three cases with consecutive consumption of green tea drinks, whose complaints of headache, vomiting, chest pain, palpitation, chest discomfort, constipation or diarrhea, and muscle pain, showed irregular electrocardiograms. Electrocardiograms were scrutinized using a digital caliper, and QT time was corrected using the Fridericia method (QTc-Fridericia= QT interval/(RR interval (sec))$^{0.33}$)[4]. Corrected QT time more than 450 ms was defined as QT prolongation.

Part 2: Analysis of residual acetamiprid in tea leaves, tea beverage, and domestic fruits

Preparation of standard solutions:

Ten mg of acetamiprid (98%, Kanto Chemicals, Tokyo, Japan) was diluted with 10 mL of acetonitrile (HPLC-grade, Kanto Chemicals) to yield a 1000 ppm standard solution, and 100, 50, and 10 ppm standard solution were prepared by sequential dilution.

Preparation of sample solutions:

Eighteen kinds of domestic fruits (six apples, three strawberries, three peaches, three Asian pears, three grapes), nine kinds of tea leaves (three Japanese, six Chinese), and three tea drinks were purchased at local market from May to October in 2006, except for three Chinese tea leaves of unknown origins and two apples that were brought by a patient. Twenty g of samples were extracted with acetone, and the extract was concentrated and redissolved in ethyl acetate followed by clean-up with a porus kieselguhr column, a magnesium silicate column, and a C18-minicolumn consecutively. Acetamiprid levels were determined using a high-performance liquid chromatograph with an ultraviolet detector (HPLC/UV). Mass spectrometry was not done.

Recovery experiment:

One mL of 100 ppm acetamiprid standard was spiked in 50 mL of acetone, and acetamiprid was recovered by the same procedure with sample preparation above mentioned. Recovery efficiency was calculated as a ratio of the detected amount and the amount spiked.

Instrumentation:

The HPLC/UV system comprised a LC-10AD pump unit, SPD-10A UV-Vis detector, and C-R6A Chromatopak integrator (Shimadzu, Kyoto, Japan) equipped with a high purity silica gel based reversed phase HPLC column, Mightysil RP-18 (150 x 4.6 (i.d.) mm, 5 μm; Kanto Chemical). The mobile phase was HPLC-grade water and acetonitrile mixture (4: 1, v/v) at a flow rate of 0.8 mL/min. Detector was set at 254 nm.

Ⅲ. Results

Part 1: Case report

The history of tea beverages that the patients consumed before the onset, clinical mani-

Footnote 1. Electronic pupilometer is a device to record the change in iris diameters in responses with photo-stimulation. After 15 min dark adaptation, pupil response wave shapes against photo-stimulus with peak wave length 605 nm, maximum photo strength 500 nW +/- 10 %, stimulus angle 15 degrees, stimulus time 0.25 sec, are recorded, analyzed and displayed by digital.

Footnote 2. Auditory brainstem responses is a record of electric potential at the brain stem obtained by the auditory nervous excitation against stimuli of short persistent click noises delivered through a headphone through the head skin. In this study stimuli frequency 10 times/sec, integral number 1000 times, filtering frequency ranges for record 100～3000 Hz. Wave shapes consist of 5～7 positive peaks which are referred to as Ⅰ～Ⅶ. Ⅰ wave represents cochlear nerve, Ⅱ wave for pontomedullary junction. Ⅲ wave for caudal pons (superior olivary nucleus), Ⅳ wave for rostral pons, Ⅴ wave for mesencephalon (mesencephalon inferior colliculi), Ⅵ wave for corpus geniculatum mediale, and Ⅶ wave for auditory cortex.

Table 1 Symptoms and examination findings of cases 1–3

	Case 1 (28 yr, female)	Case 2 (31 yr, male)	Case 3 (51 yr, female)
History of tea beverage intake	>500 mL/day, >3 months	600 mL/day, 3 months	1000–1500 mL/day, >3 months
History of pesticide exposure	no	underfloor termite extermination (chlorpyrifos, imidacloprid)	glufosinate inhalation before onset
Past clinical history	no	depression for 4 yr	neonicotinoid intoxication-like symptoms 3 weeks ago
Subjective symptoms	chest pain, headache, sleepiness, constipation, throat pain, muscle pain	chest pain, palpitation, headache, nausea, depression, sleepiness, nightmare, restlessness, short-term memory impairment, muscle pain, muscle weakness	chest pain, palpitation, headache,, nausea, muscle pain, muscle weakness
Clinical findings	height 159 cm, weight 56 kg, BP 130/92, temperature 38.5 C, SpO2 97%, muscle spasms throughout the extremities, impaired short-term memory, impairment of speech	height 176 cm, weight 56 kg, BP 120/78 temperature 38.5 C, SpO2 97%, finger tremor, difficulty in communication	height 155 cm, weight 60 kg, BP 150/80, finger tremor
Electrocardiography findings	sinus tachycardia, ST depression	ST elevation, right intraventricular conduction delay	sinus tachycardia, T wave flattening
Auditory brainstem response findings V wave latency I-V interval	 5.59/5.56 ms 4.13/4.14 ms	 5.66/5.61 ms 4.04/4.20 ms	 5.17/5.20 ms 3.74/3.77 ms
Pupillography findings	early miotic pupil, shortening of dilatation time	early miotic pupil, pupillary miotic rate elevation	early mydriasis, pupillary miotic rate elevation

Table 2 Pupillography findings of cases 1–3

	Case 1		Case 2			Case 3	
date of examination	the first visit		the first day	after 10 weeks	after 15 weeks	the 7th day after the re-visit	
pupil responses	direct	indirect	direct	direct	direct	direct	indirect
D1: dark adaptation pupil diameter (mm)	6.2↓	6.1↓	5.7↓	5.9↓	5.9↓	7.3↑	7.4↑
A1: dark adaptation pupil area (mm2)	27.3↓	27.0	25.8↓	26.6↓	26.7↓	40.6↑	40.6
D2: after photic stimulation pupil diameter (mm)	4.5	4.9	4.4	4.8	4.8	5.1	5.4
CR: miotic rate	0.27↓	0.19↓	0.24↓	0.19↓	0.18↓	0.30↓	0.26↓
t1: latency (ms)	349↑	333↑	304↑	300↑	333↑	283↑	266
t2: pupil 1/2 miotic time (ms)	650↑	283↑	384↑	183	183	317↑	317↑
t3: pupil miotic time (ms)	1084↑	617	850↓	500↓	617↓	1100↑	1084↑
t5: 63% dulation time (ms)	1150↓	1283↓	1800	1666	1666	2733↑	1733↑
Vc: maximum contraction velocity (mm/s)	3.6	3.7	5.1↑	5.1↑	4.1	5.1↑	5.1↑
Vd: maximum dilation velocity (mm/s)	2.3↑	2.0	1.6	1.2↓	1.2↓	1.6	2.0

festation, and laboratory results are shown in Table 1. Details of pupillography finding are shown in Table 2.

Case 1: Twenty eight-years-old woman who recovered in short time after the diagnosis and treatment

She had been healthy and had been consuming commercial green tea beverage in a PET bottle more than 500 mL/day for three months or more. In early August 2007, she visited A-clinic with multiple subjective symptoms including anterior chest pain that had started on the preceding day.

When she visited, her clinical manifestations were mild hypertension (138/90 mmHg), severe tachycardia, high fever, muscle spasms throughout the extremities, impaired short-term memory (poor recall of contents of the previous day's meal), and impairment of speech accompanied with disturbed consciousness (hardly able to speak with absence of mind). The patient's objective findings are as follows:

- Urine examination: ketone body (+++), protein (-), glucose (-)
- Blood-examination finding: red bood cells count 4.71 x $10^6/\mu$l, Hb 14.2 g/dl, Ht 44.1 %, white blood cell count 10,250/μl (high),

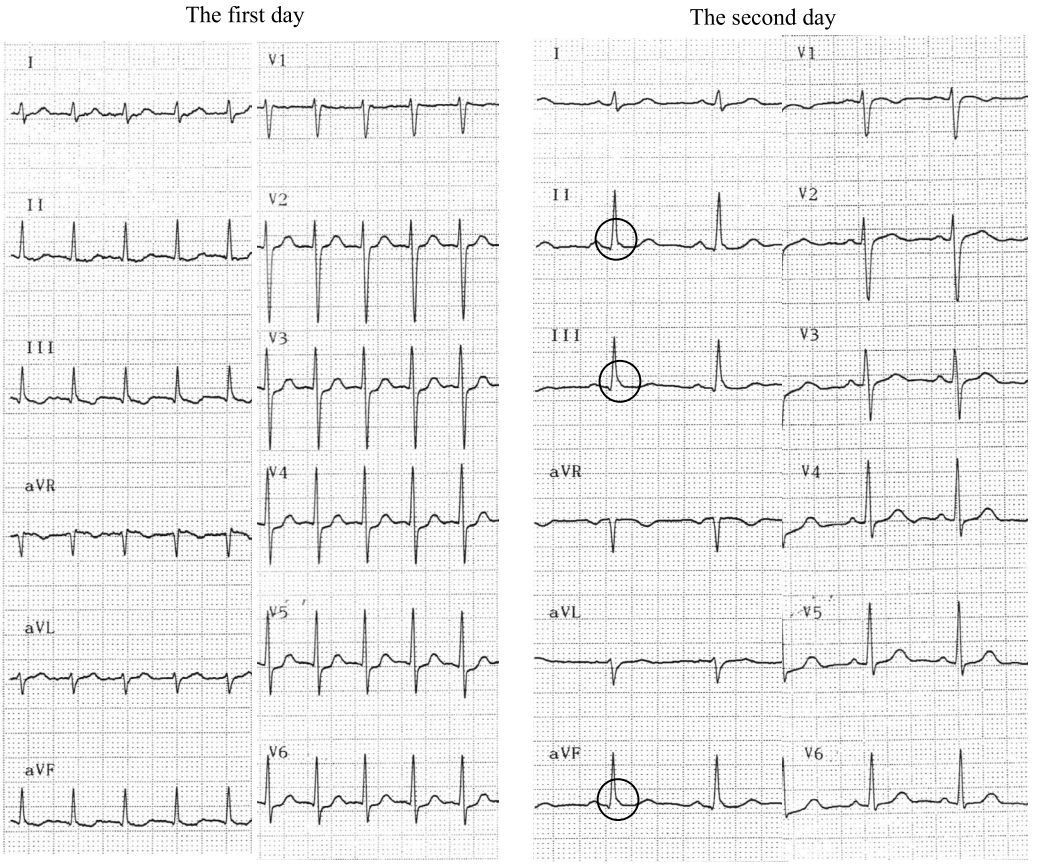

Fig. 1 Electrocardiograms in case 1

On the first visit (left), BP 138/90, sinus tachycardia, HR 146 bpm, ST depression in Ⅱ, Ⅲ, aVF; on the following day (right), BP 100/58, HR 77 bpm, corrected QT time 413 ms, S wave slur (flattening of S wave part of QRS, circle) in Ⅱ, Ⅲ, aVF.

differential count of leucocytes: neutrophil 82.2 %, lymphocyte 11.7 %, CPK 57 IU/l (normal), CRP 0.97 mg/dl (up)

- Electrocardiography findings (Figure 1): sinus tachycardia and ST depression in II, III, and aVF
- Pupilography finding (Table 2): early miotic pupil and shortening of dilation time
- Auditory brainstem response (ABR): shortening of V-wave latency, shortening of I-V peak interval
- Treatment: infusion (maltose-Ringer's solution, glutathione), medication (antibiotics, nonsteroidal anti-inflammatory drugs, glutathione, glucronolactone, lactobacillus), prohibition of green tea intake

The patient's subjective symptoms improved on the following day, blood pressure returned toward normal (100/58), and electrocardiogram showed a normal heart rate and appearance of slur in S-wave segment of QRS wave in II, III, and aVF.

Case 2: Thirty one-year-old man who recovered in six weeks after the diagnosis and treatment

He had been healthy and had been working as a company employee. He used chloropyrifos (organophosphorus pesticide) in 1998 and imidacloprid (neonicotinoid pesticide) in 2003 under the floor of his house for extermination of termites. Since 2003 he manifested depression, was diagnosed with clinical depression, and prescribed psychotropic drug by a neighborhood doctor. However, his symptoms persisted; and he went on medical leave.

He had been consuming commercial green tea beverages more than 600 mL/day from January 2007. Since early March 2007, he displayed muscle pain, tremor, general fatigue, impaired memory, insomnia, restlessness, disturbance in conversation, palpitation, and chest pain. His I-V peak interval tended to shorten by the ABR recorded by the psychiatrist. Due to suspected intoxication, he visited A-clinic with a referral.

In early April 2007 at the first visit, his clinical manifestations were normal blood pressure (120/78 mmHg), high fever, finger tremor, and the inability to make a conversation and difficulty in communication. The patient's objective findings are as follows:

- Electrocardiographic findings (Figure 2): heart rate 67 ppm, ST elevation in I, AVL, and V4-6 leads
- Pupilography finding on the seventh day after the first visit (Table 2): initial miotic pupil, pupil miotic rate elevation.
- Treatment: termination of psychotropic drug, infusion (maltose-Ringer's solution, glutathione), medication (antibiotics, small dose steroid with anti-histamine drug, glutathione, glucronolactone, lactobacillus, bromazepam (benzodiazepine), butropium bromide (anticholinergic drug), ketotifen (antiallergic drug)), prohibition of green tea intake

Along with treatment early miotic pupil improved slightly, although persisted for fifteen weeks, and pupil miotic rate elevation returned to normal. Shortening of pupil dilatation rate appeared in the tenth week after the first visit and persisted for fifteen weeks.

The patient improved subjective symptoms gradually. On the sixth week he improved conversation ability to communicate with family members, enriched his emotional expression, and his restlessness disappeared. His electrocardiographic ST elevation improved on fifteenth week after the first visit.

Case 3: Fifty-one-year-old woman who recovered by treatment but her symptoms recurred upon the exposure to herbicide

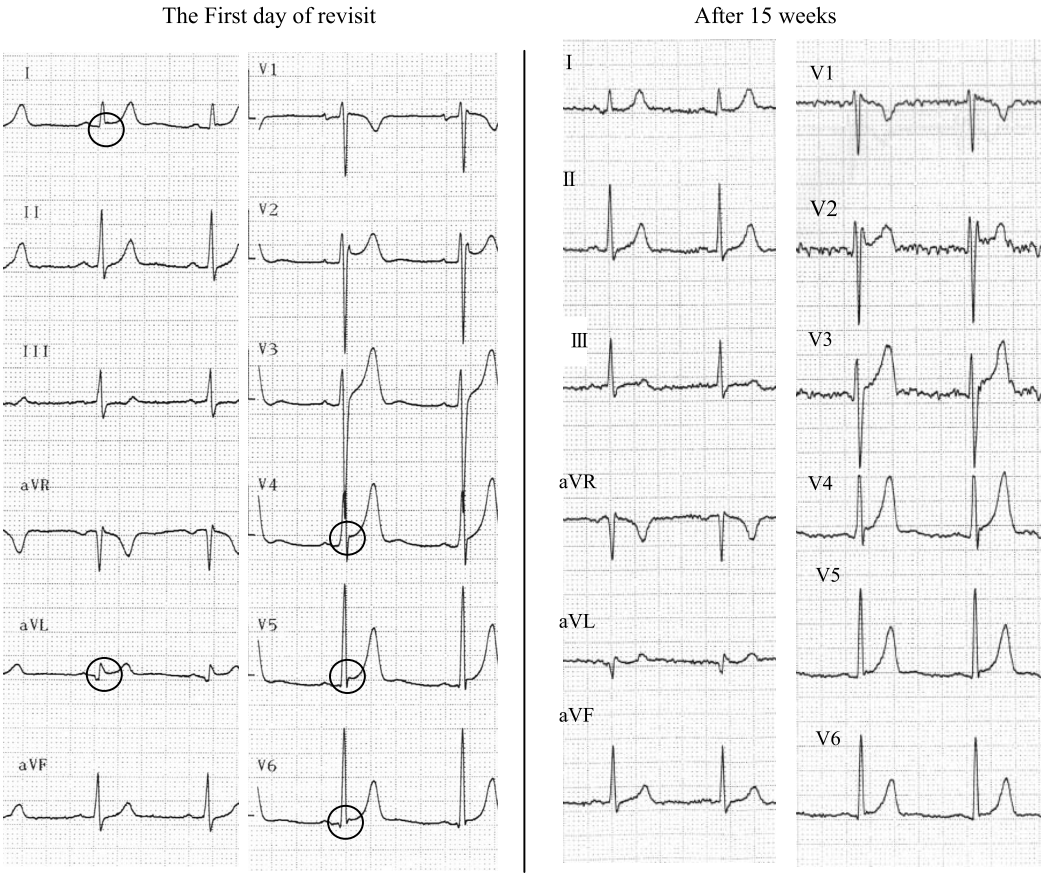

Fig. 2 Electrocardiograms in case 2

On the first visit (left), AVL, ST elevation in V2-6, right ventricular conduction delay; fifteen week after the first visit (right), contamination of electromyogram was observed, ST elevation with S wave slur (circle) in Ⅰ and aVL, improved ST elevation in V5 and 6, right ventricular conduction delay remained.

She had been healthy and had been consuming green tea beverages 1000-1500 mL/day for three months or more. In mid May 2007, she visited A-clinic with general fatigue and tremor. Her symptoms improved with the prohibition of green tea intake and medication (glutathione, glucronolactone, lactobacillus, and benzodiazepine). The patient's ABR finding on the fifth day after the first visit were shortening of V-wave latent time and shortening of I-V peak interval.

In early June 2007, after her family member sprayed herbicide, glufosinate, on the neighboring land to her house, she felt nausea, headache, and dizziness. And few days later she had chest pain so she visited A-clinic again. She had mild hypertension (150/80 mmHg) on her second visit. The patient's objective findings are as follows:

- Electrocardiographic findings (Figure 3): heart rate 87 ppm, ST depression and T wave flattening in II, III, AVF, and V3-6, adjusted QT prolongation
- Pupilography finding on the seventh day after the second visit (Table 2): early mydriasis, pupil miotic rate elevation
- Treatment: infusion (maltose-Ringer's solution 200 ml, glutathione 100 mg) four

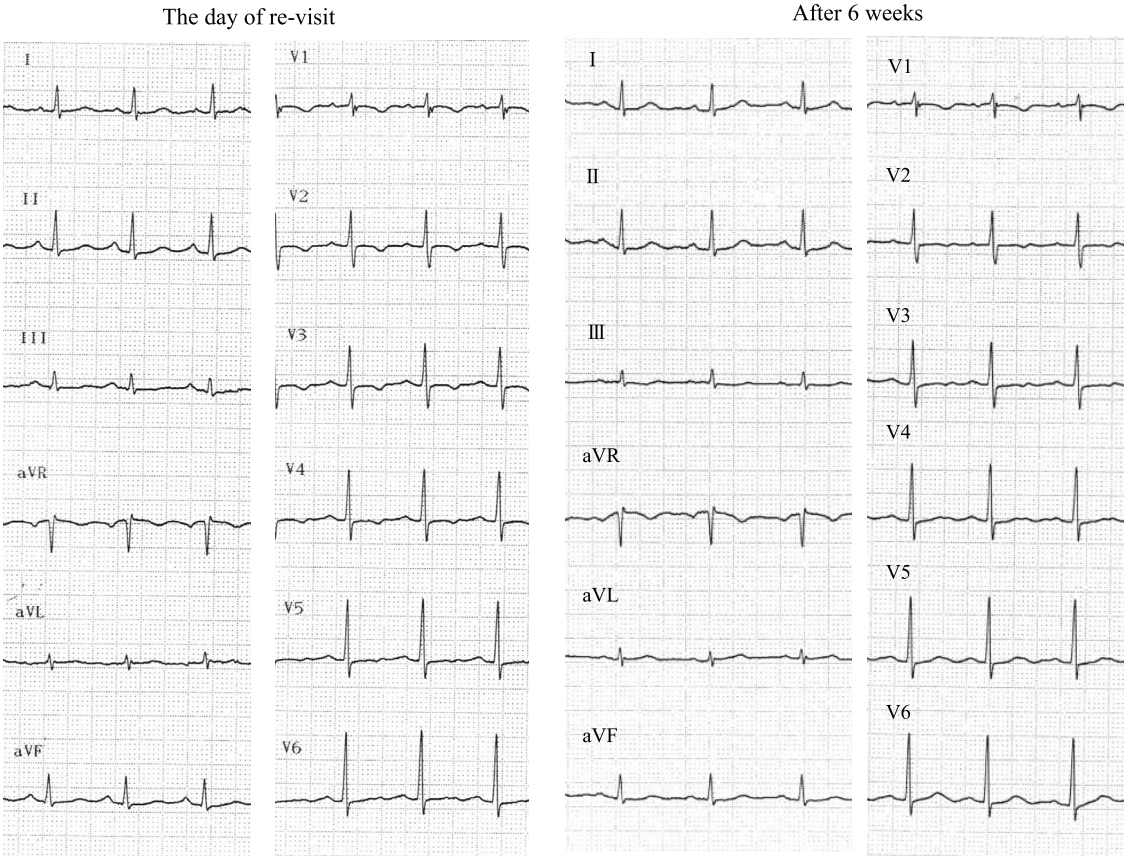

Fig. 3 Electrocardiograms in case 3

On the re-visit (left), QT prolongation and flattening of T were observed; six weeks after the re-visit (right) they were improved.

times in sixteen days.

Her symptoms improved gradually. Electrocardiographic ST change improved in six weeks and QT prolongation returned toward normal in two weeks.

Part 2: Residual acetamiprid analysis in tea leaves, tea beverages, and domestic fruits

The retention time of acetamiprid was 10.44 min under HPLC conditions, and the recovery efficiency was 69.8 %. The result of acetamiprid analysis is shown in Table 3. Detectable residual acetamiprid levels were 10.7 +/- 0.0 ppm in a Chinese green tea leaves, 19.9 +/- 2.1 ppm in Chinese oolong tea leaves, and 4.88 +/- 0.11 ppm in a domestic apple core that is brought by a patient of A-clinic. All of the detected levels were below the Maximum Residual Levels (MRLs). The patient who brought apple E (Table 4) had been eating apples from the same box everyday, and visited A-clinic with a severe migraine. Residual acetamiprid was detected in a domestic green tea beverage at a level of 2.45 +/- 0.34 ppm. According to the manufacturer of the tea beverage, they didn't have any contract regarding the use of acetamiprid with tea leaf producers, and they confirmed that residual pesticides levels in tea leaves were below the MRLs, which were determined by sampling and testing when tea leaves were purchased.

Ⅳ. Discussion

Green tea contains flavonoids such as catechins, caffeine, vitamins B_2, B_6, C, E, and K, amino acids, sugars, organic acids, carotene, chlorophyll, saponin, and minerals (K, Ca, Al, Mn, Zn). There have been reports on the adverse health effects of drinking tea beverages caused by catechins and caffeine.

Table 3 Acetamiprid levels in tea leaves, tea beverages, and domestic fruits

sample	detected level (ppm)	MRL (ppm)
Chinese tealeaves A*	not detected	50
Chinese tealeaves B*	19.88 +/- 2.07	50
Chinese tealeaves C*	10.72 +/- 0.00	50
Chinese tealeaves D	not detected	50
Chinese tealeaves E	not detected	50
Chinese tealeaves F	not detected	50
Domestic tealeaves A	not detected	50
Domestic tealeaves B	not detected	50
Domestic tealeaves C	not detected	50
Green tea beverage A	2.49 +/- 0.34	NA
Green tea beverage B	not detected	NA
Green tea beverage C	not detected	NA
Apple A (meat)	not detected	5
Apple B (meat)	not detected	5
Apple C (meat)	not detected	5
Apple D (meat)	not detected	5
Apple D (core)	not detected	5
Apple E (meat) †	not detected	5
Apple E (core) †	4.88 +/- 0.11	5
Apple F (meat)	not detected	5
Apple F (core)	not detected	5
Strawberry A	not detected	5
Strawberry B	not detected	5
Strawberry C	not detected	5
Peach A	not detected	5
Peach B	not detected	5
Nectarine	not detected	5
Asian pear A	not detected	5
Asian pear B	not detected	5
Asian pear C	not detected	5
Grape A	not detected	5
Grape B	not detected	5
Grape C	not detected	5

(*) indicates unknown origin (†) indicates the specimen was brought by a patient

Catechins have health-promoting effects, such as the suppression of carcinogenesis, plasma cholesterol elevation, blood pressure elevation, and blood glucose elevation[5]. Tea beverages supplemented with catechins are registered as "Food for specialized health use" in Japan. However, there are reports of cases who had severe liver dysfunction caused by the consumption of beverages concentrated with catechins and received a liver transplant in the US and Canada[6].

Caffeine is a type of plant alkaloid, methylxanthine. Caffeine is a non-specific blocker of adenosine receptors, and exhibits a stimulant effect via antagonizing adenosine receptors in striatal neurons that are projected to the basal ganglia of the brain[7,8]. Caffeine also increases the serotonin levels in the brain[9], but there is previously no report on serotonin syndrome after consecutive green tea intake. Caffeine increases intracellular cAMP levels by non-selective inhibition of phosphodiesterase, followed by sympathetic excitation effects, such as the increase in the force of myocardial contraction, relaxation of bronchial smooth muscle, contraction of cerebral arteriole, and stimulation of gastric acid secretion[10]. Caffeine increases high-frequency power (HF) by heart rate variability analysis, that is, increases parasympathetic tone[11]. Caffeine increases plasma epinephrine levels[12–14]. The over consumption of caffeine promotes excretion of urinary Ca, Mg, and Na[15]. Half lethal dose (LD_{50}) of caffeine is approximately 200 mg/kg, and over 10–12 g of caffeine intake is considered dangerous for adults. Elimination half-life in vivo is approximately 3.5 hr. Clinical manifestations of caffeine over consumption are

reportedly insomnia, restless, frequent urination, over irritability, tremor, hypertension[16], tachycardia[17], and onset or degenerating of panic disorder in short term[18], and mental confusion, palpitation, restlessness, insomnia, and memory disturbance in long term[19]. Caffeine content in green tea leaves is approximately 3 % (w/w), and therefore the amount of caffeine containing in 500 ml of green tea beverage is estimated to be approximately 150 mg, assuming 1 % (w/v) of tea leaves is used for the beverage.

Several pesticides, which remain in green tea leaves and possibly cause nicotinic receptor related symptoms, are neonicotionids, organophosphates, and carbamates. Acetamiprid is a water-soluble neonicotinoid insecticide, and highly permeable from soil to plants[20]. Acetamiprid has a longer elimination half-life in vivo than other neonicotinoid insecticides, and tends to accumulate in the brain. In an ip-treated mice experiment, half-lives of acetamiprid in brain, liver, and plasma were more than 4 hr, and total elimination in 24 hr after administration including metabolites was only 13 %[21]. Acetamiprid was partially metabolized in plants and a des-cyano metabolite was formed during the plant metabolism[22].

Acetamiprid has a selective agonist of $\alpha 4\beta 2$ subtype nicotine receptor, and its sensitivity of insect receptor is 84-fold higher than the human receptor[23]. Human $\alpha 4\beta 2$ nicotine receptor exists mostly in the brain and is related to nicotine dependence; therefore a partial antagonist, varenicline, is commercialized as a therapeutic for smoking cessation[24]. As the side effect of varenicline, anger, depression, and suicidal ideation were reported in the US[25]. There are several other subtypes of nicotine receptors inside and outside of the brain (Table 4). Orally acetamiprid-treated rats exhibited symptoms in the central nervous system, such as a decrease in locomotor activity, reduced alertness, and convulsion, symptoms related to autonomic ganglion and neuromuscular junctions, such as muscle weakness, decrease in pupil reflex, tremor, and pupillary

Table 4 Presumed modes of action and effector sites of acetamiprid

Mode of action	nicotinic acetylcholine receptor (nAChR) agonist				cyanide intoxication
Effector site	Brain	Autonomic ganglion	Neuromuscular junction	Lymphocyte	cell respiration/ cytochrome c
Sub-type	$\alpha 3\beta 4$, $\alpha 4\beta 2$, $\alpha 7$	$\alpha 3\beta 4$, $\alpha 3\beta 2$	$\alpha 1\beta 1\varepsilon\delta$	$\alpha 7$	
Ligand	parent compound, metabolites	metabolites	metabolites	metabolites	cyanide
Symptoms	insomnia, sleepiness, restlessness, short-term memory impairment	constipation, diarrhea, headache, nausea, vomitting, palpitation, chest pain, angor pectoris, hypertension	muscle pain, tremor, muscle weakness, general malaise, neck stiffness	fever, bacterial infection, viral infection, airway inflammation	headache, vomitting, weakness, pallor, edema, tremor, abnormal plasma glucose, achromatopsia
ECG findings	QT prolongation, ST change	tachycardia, bradycardia, arrhythmia	electromyogram		ST change, intraventricular conduction delay, low potential

dilatation, and oliguria[26], which resembled those of cyanide intoxication. Because these symptoms were observed in rats orally treated with acetamiprid metabolites[26], it is conceivable that acetamiprid and its metabolites interact with nicotine receptors other than human $\alpha 4\beta 2$ subtype, and result in autonomic and neuromuscular manifestation, and possibly symptoms of intoxication of cyanogens formed during the acetamiprid metabolism.

In this study residual acetamiprid was suspected in two kinds of tea leaves produced in China. The MRL of acetamiprid is 2ppm for tea leaves in China[27]. Although acetamiprid was not detected in all of the domestic tea leaves, residual acetamiprid was suspected in a kind of green tea beverage, which was produced from only domestic tea leaves. In Japan the application of acetamiprid on tea leaves is restricted during only a limited time, 14 days prior to harvesting; and the MRL of acetamiprid is 50 ppm for tea leaves. However, there are several reports; acetamiprid was not detected in tea leaves, which were grown with the application of acetamiprid, several days after harvesting[28]. Plants metabolized acetamiprid and many metabolites had strong bioactivities[22]. A relatively high level of residual acetamiprid was suspected in the core of apple in this study. Taking everything into account, it is plausible that acetamiprid metabolites had accumulated and were related to the onset of manifestation of the patients.

Regulation of acetamiprid by the Japanese administration is currently under consideration by the food safety committee, who conducted its reassessment in 2008, based on a two-generation reproduction test and a two year chronic toxicity/carcinogenicity tests, and set the Acute Reference Dose (ARfD) 0.1 mg/kg/-day newly, in addition to the No Observable Adverse Effect Level (NOAEL) 7.1 mg/kg weight/day and the Acceptable Daily Intake (ADI) 0.071 mg/kg/day. The MRLs of acetamiprid for foods in Japan as of in November 2008 are set higher than those in EU and the US (Table 5). The MRLs in Japan are calculated based on average daily intake in the National Nutrition Survey, if a person eats food with a low average daily intake, for example average daily intake of grape is 5.8 g/day, contradictorily he or she will be exposed to acetamiprid as high as ADI. It is a discrepancy that when a person eats three bunches of grapes containing acetamiprid at the MRL of 5 ppm, the amount of acetamiprid intake is almost the same as its ADI. From the viewpoint of legal consistency, the authors think that the MRLs of acetamiprid need to be revised.

Although the residual levels of organophosphorus and carbamate pesticides in tea leaves and tea beverages need to be examined, if they are the causes of these cases considerable amounts should be contained.

If these cases were caused by chronic overconsumption of caffeine or catechins, there are previously unreported manifestations as follows: case 1, throat pain, high fever, muscle pain, urinary ketone (+++), increase in neutrophil, decrease in lymphocyte, CRP elevation, miotic pupil, abnormality of pupillary reflex, S wave slur; case 2, high fever, muscle weakness, chest pain, ST elevation, S wave slur, miotic pupil, abnormal papillary reflex; case 3, ST depression, QT prolongation, dilatation of pupil, and abnormality of pupillary reflex.

Case 1 visited the doctor with a high fever, tachycardia, and muscle spasms, which resemble with the serotonin syndrome. However the dilation of pupil was not shown but early miotic pupil and shortening of dilation time was observed by the pupil reaction. Case 1 was

Table 5 Comparison of the maximum reference levels (MRLs) of acetamiprid in Japan, the US, and the EU.

	Japan	US	EU
Tea (dried leaves)	50		0.1*
Onion	5	0.02	0.01*
Grapes	5	0.2	0.01*
Plums	5	0.2	0.02
Eggplants	5	0.2	0.1
Green peppers	5	0.2	0.3
Tomatoes	5	0.2	0.1
Cucumbers	5	0.5	0.3
Lemons, Oranges, Grapefruits, Other citrus	5	0.5	1
Strawberries	5	0.6	0.01*
Peas	5	0.6	0.01*
Apples, Asian pears, Pears	5	1	0.1
Apricots, Peaches	5	1.2	0.1
Cherries,	5	1.2	0.2
Cabbages, Cauliflowers, Broccoli,	5	1.2	0.01*
Celeries, Spinaches	5	3	0.01*
Lettuces, Parsleys,	5	3	5
Leeks	5	4.5	0.01*
Mask melons	2	0.5	0.01*
Pumpkins	2	0.5	0.01*
Melons	1	0.5	0.01*
Watermelons	0.5	0.5	0.01*
Potatoes	0.5	0.01	0.01*
Garlic	0.2	0.02	0.01*
Gingers	0.1	0.01	0.1*

(*) Indicates lower limit of analytical determination

predominantly controlled by parasympathetic nerve with both sympathetic and parasympathetic tones, which are conceivably caused by the stimulation of autonomic ganglion by neonicotionid. ST depression followed by S wave slur is interpreted as myocardial impairment, which may be induced by cyanide formed by metabolizing acetamiprid.

Case 2 was prescribed with a psychotropic drug for depression, which emerged upon chronic organophosphorus and neonicotinoid exposure, and exhibited a high fever but not focal infection finding like in case 1. Other complaints were skeletal muscle symptoms and chest pain, and early miotic pupil but shortening of pupil contraction time were seen by pupillography. Patients with chronic organophosphorus intoxication usually exhibit miotic pupil and delayed pupil responses in dilation and contraction[29]. Although it is possible that the agonistic effect of neonicotinoid on acetylcholine receptors may be augmented by the inhibition of acetylcholinesterase on the chronic exposure of organophosphorus compounds or carbamates, the symptoms of case 2 are not explainable by the direct organo-

phosphorus effect. The evaluation of the autonomic functions of patients who had been administered psychotropic drug should be performed carefully, however the termination of the psychotropic drug and the treatment focusing on detoxication resulted in the improvement in neuropsychiatric symptoms and pupil responses. In the early stage of the disease ST elevation followed by S wave slur was observed by electrocardiography. Although the determination of thiocyanate ion in urine was not performed, it may be caused by cyanide as well as Case 1.

Case 3 exhibited acetamiprid intoxication-like symptoms, which were emerged after consecutive intake of green tea beverage and were once relieved by the treatment, but acute exacerbation occurred upon the exposure of glufosinate inhalation. It happened more than one month after the first visit, therefore it falls into the chronic stage. The electrocardiography findings were ST depression accompanied with QT prolongation, which are possibly susceptible with ischemic heart disease, myocarditis, or pericarditis, however, the treatment focusing on detoxication resulted in the improvement of electrocardiography findings and chest pain. Pupillography findings were early papillary dilation and pupil miotic rate elevation, which was predominantly controlled by the sympathetic nerve and was different from cases 1 and 2, however; deterioration of the parasympathetic function was not considered. Although glufosinate can cause severe sinus bradycardia upon high dose of oral exposure[30], there is no report on acute cardiovascular manifestation caused by inhalation of glufosinate alone. It is thought that glufosinate inhibits glutaminate synthetase and glutaminate carboxylase to lead to the reduction in glutamate levels in the central nervous system, causing drowsiness, memory impairment, and convulsion[31, 32]. Glutaminate and nicotine receptors are co-localized at the terminals of adrenaline neurons in the brain[33], therefore it is conceivable that the reduction in the glutamate level and nicotine receptor stimulated by acetamiprid changed adrenal neuron tone, resulting in sympathetic tone.

In all of the cases 1–3, shortening of I–V peak interval was observed by ABR. Because it has been reported that V wave latent time and the shortening of I–V peak interval was observed by ABR with a high dose of caffeine exposure[34], whether the shortening of I–V peak interval is indicative to neonicotinoid intoxication needs to be further investigation.

Ⅴ. Conclusion

Acetamiprid can remain in commercial domestic foods, and the intake of residual acetamiprid from food, which contains acetamiprid below the MRLs, can exceed the ADI. In cases of patients who consumed tea beverages consecutively, we observed clinical findings that cannot be explained by caffeine consumption, alone, such as headache, nausea, palpitation, chest pain, muscle weakness, impaired short-term memory, and verbal disorder, as well as sub-acute tachycardia, abnormality of ST-segment, deformation of QRS wave shape, and QT prolongation by electrocardiography, and sympathetic and parasympathetic tone by pupillography. There is still a possibility that residual neonicotinoid especially acetamiprid in food is related to the onset of sub-acute and chronic neuromuscular disorder, central nervous system damage, myocardial impairment, and disorder of ocular accommodation.

Remark: The result of part 1 of this study was

presented at the 27th Pupil Colloquium, Hamamatsu, Japan in 2007.

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要約

茶飲料、果物、野菜ジュースの連続摂取後に、頭痛、吐気、めまい、動悸、胸痛、振戦、筋痛、筋脱力、記憶障害などのニコチン様アセチルコリン受容体関連症状と共に尿中 SCN 高値を示す症例が、近年増加している。シアン基を有しニコチン様アセチルコリン受容体アゴニストである殺虫剤アセタミプリドの食品残留基準は諸外国に比し高い。我々は緑茶飲料を毎日500ml 以上連続摂取し同症状を訴え受診した患者３例の心電図、聴性脳幹反応、瞳孔反応を検討した。心電図に洞性頻脈、ST 変化、QT 延長、聴性脳幹反応にⅠ-Ⅴ peak interval の短縮、瞳孔反応に初期縮瞳または散瞳と対光反応亢進がみられ、交感、副交感両方の刺激状態が示唆された。あわせて茶葉、茶飲料、国産果物のアセタミプリド濃度を HPLC/UV 法で計測したところ、中国産茶葉２種、茶飲料１種、国産りんご１種の芯において、それぞれ19.88+/-2.07 ppm、10.72+/-0.00ppm、2.49+/-0.34ppm、4.88+/-0.11ppm（平均+/-SD）であった。

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