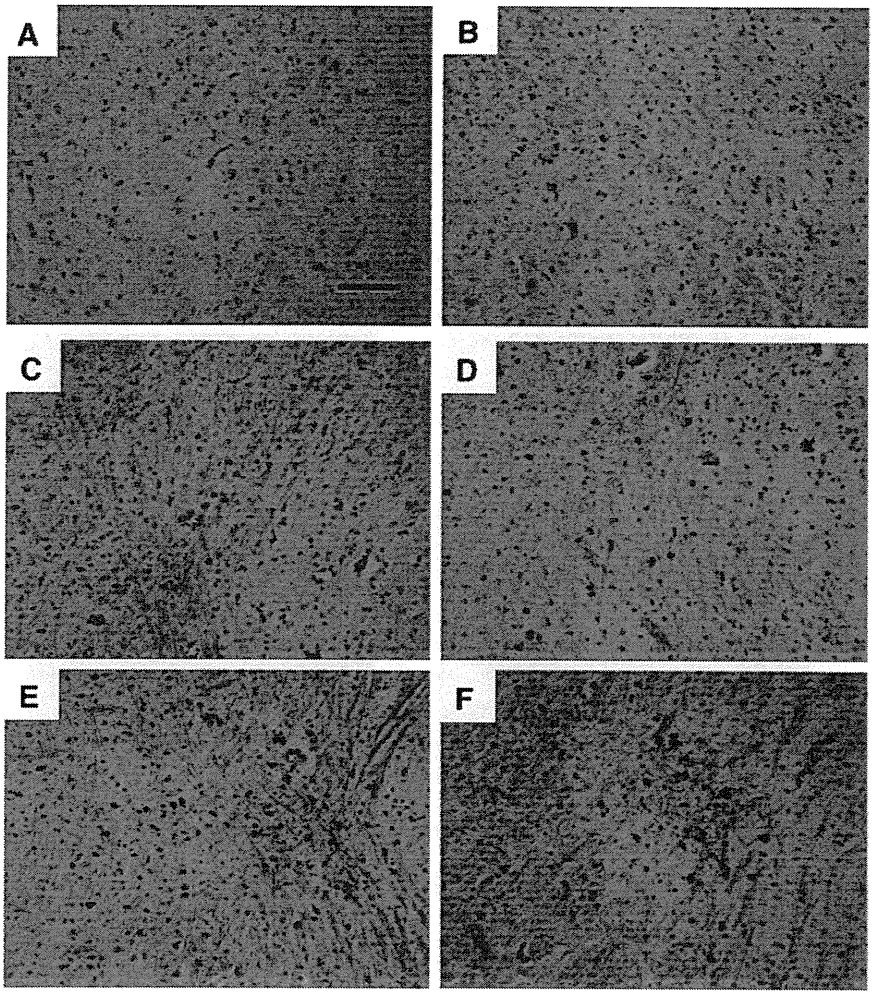

Fig. 5 – Images of S100β-positive astrocytes with DAB immunostaining in the substantia nigra counterstained with hematoxylin (1 week after MPTP-treatment): labelings and size of bar scale are same with those in Fig. 3.

in the number and area of TH-positive dopamine neurons (Figs. 2A, 2B, and 3). From these findings, it is suggested that zonisamide shows weak MAO-B inhibition on MPTP-induced cytotoxicity.

In addition, consistent with a previous report (Yabe et al., 2009), our data of 1 week also showed that zonisamide pre-treatment increased striatal dopamine turnover of MPTP-treated mice (Fig. 1B). Due to the inhibitory effect of zonisamide on MAO-B, relatively more intact TH-positive dopamine neurons remained in zonisamide pre-treated mice. However, zonisamide-mediated MAO-B inhibition is reversible and incomplete (Sonsalla et al, 2010). Thus, the inhibitory effects of zonisamide with single dose on MAO-B may not exist after several days, while the effect of administered MPTP with multiple dose (15 mg/kg×4) may be continued to several days and affect dopamine neuronal productivity. Together with these possible events, it could be suggested that, at early period after zonisamide administration, relatively more dopamine was produced by rescued TH-positive neurons which turned on 7th day increased dopamine turnover. Whereas, zonisamide post-treatment was ineffective in increasing dopamine turnover (Fig. 1B) and dopamine neurons might already be damaged by MPTP before zonisamide was administered.

Notably, zonisamide, when administered alone, increased the dopamine content compared to normal control. On the basis of these observations, it is likely that zonisamide increased the contents and activity of TH-protein of dopamine neurons. Some other reports also supported this possible mechanism of zonisamide (Yano et al, 2009; Yokoyama et al, 2010). Indeed, 9 weeks after MPTP-treatment, zonisamide attenuated the depletion of striatal dopamine contents (Fig. 8A) and, conversely, in zonisamide-treated group, the dopamine turnover of MPTP-treated dopaminergic neurons were gradually decreased (Figs. 7B and 8B). This finding has led us to speculate that an increase of dopamine production by zonisamide-mediated cured dopamine neurons may decline dopamine turnover. Together with these findings, it also can be assumed that zonisamide helps TH-positive dopamine neurons to recover and it takes several weeks for complete recovery as a previous study reported that dopaminergic neurons that survive MPTP lesioning increase TH-protein and may repopulate the striatum with axonal growth and

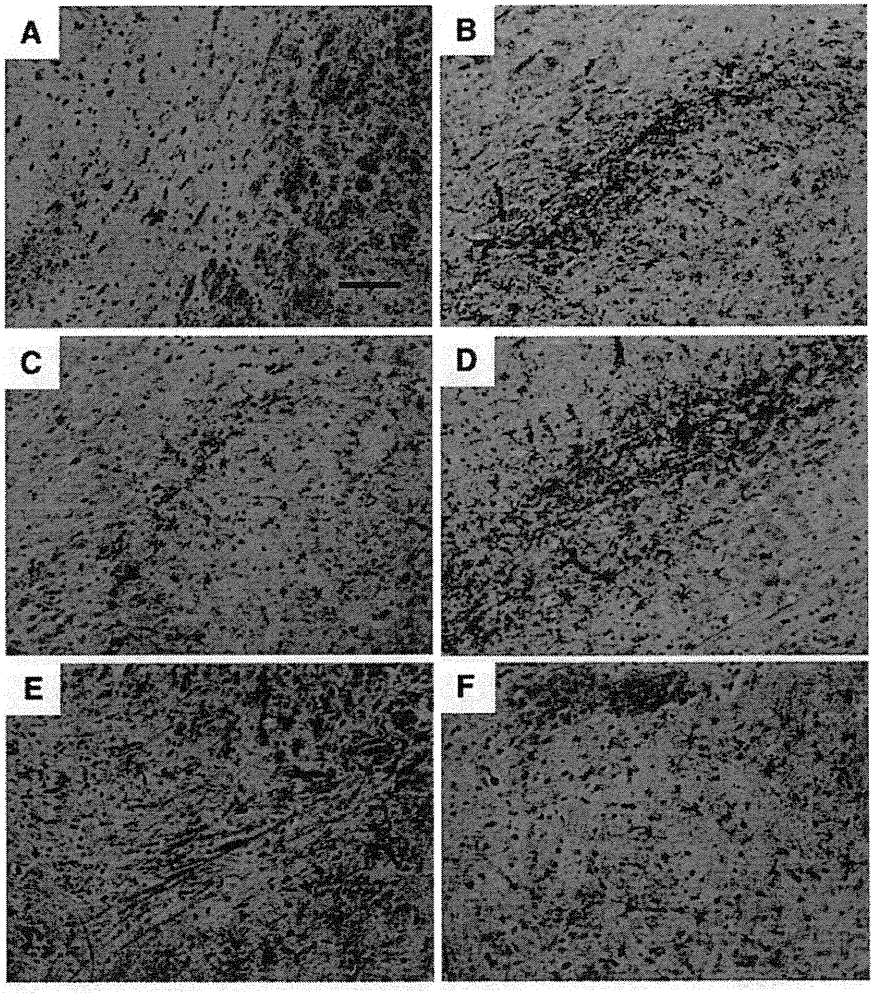

Fig. 6 – Images of GFAP-positive astrocytes with DAB immunostaining in the substantia nigra counterstained with hematoxylin (1 week after MPTP-treatment): labelings and size of bar scale are same with those in Fig. 3.

branching, indicating neuroplasticity (Jakowec et al., 2004). Therefore, our present findings suggest that therapeutic strategies targeted at the activation of TH activity with zonisamide may offer a significant potential for restoring the functional capacity of the surviving dopaminergic neurons in individuals affected with Parkinson's disease.

Astrocytes play an important role in maintaining the homeostasis of the neuronal microenvironment, such as ion homeostasis, uptake of neurotransmitters, and the formation of blood–brain barrier (Walz, 1989; Nicholls & Attwell, 1990; Brightman, 1991). Astrocytes are activated in response to neuronal damage and these activated astrocytes may help neurons to recover from neuronal damage (Kato et al., 1994; Ridet et al., 1997). In addition, S100β is primarily secreted by a subtype of mature astrocytes in MPTP-treated mice (Himeda et al., 2006) and extracellular S100β exerts autocrine effects that promote astrocyte proliferation (Selinfreund et al., 1991; Donato, 2003). S100β has neurotrophic actions, including neurite outgrowth, neuron survival, and protection against glucose deprivation and excitotoxicity (Van Eldik & Wainwright, 2003). It might be also involved in cellular defense mechanism against oxidative stress (Migheli et al., 1999). Therefore, we next considered to study the involvement of astrocytes in zonisamide-mediated neuroprotection against MPTP-toxicity. Consistent with a previous report (Asanuma et al., 2010), we found that treatment with zonisamide increased the number of S100β-positive astrocytes in the substantia nigra of MPTP-treated mice at 1 week. This represents another important mechanism whereby zonisamide may be neuroprotective as S100β have been associated with trophic effects. By contrast, zonisamide pre-treatment 1 h prior to MPTP and zonisamide post-treatment 1 h after MPTP did not increase the number of GFAP-positive astrocytes where a few damage to substantia nigra induced by MPTP may only activate S100β-positive astrocytes but not GFAP-positive astrocytes, thus allowing recovery of dopamine neurons. In addition, post-treatment with zonisamide 1 day after MPTP resulted in more dopaminergic neuronal cell death and increased the number of GFAP-positive astrocytes. Thus, MPTP-treated dopamine neuronal cell death causes astrocytes to aggregate in the substantia nigra which is enhanced by the administration of zonisamide. From this, it can be assumed that zonisamide post-treatment 1 day after MPTP increased both GFAP-positive and S100β-positive astrocytes where GFAP-positive

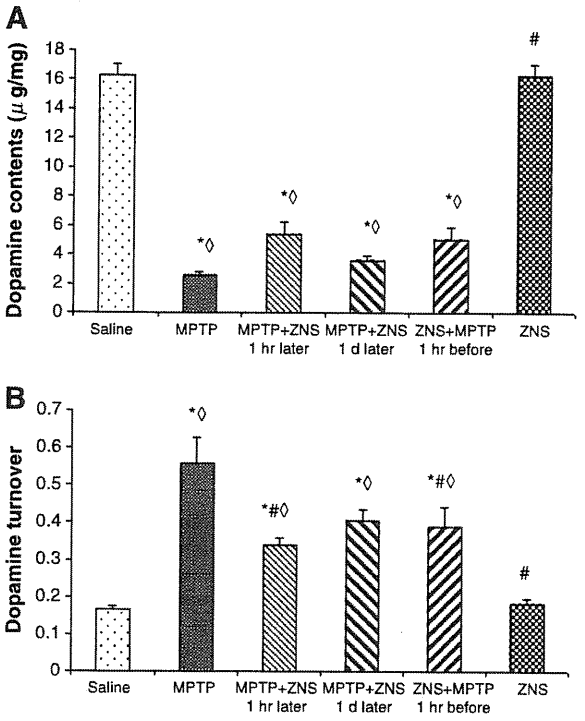

Fig. 7 – Effects of zonisamide administered 3 weeks after MPTP on (A) dopamine contents and (B) dopamine turnover in striatum. Values are presented as the mean ± SEM (n = 6–10 per group). The levels of significance were analyzed by ANOVA, with *post hoc* Tukey test: sequence of groups and symbols for statistical analysis are same with those in Fig. 1.

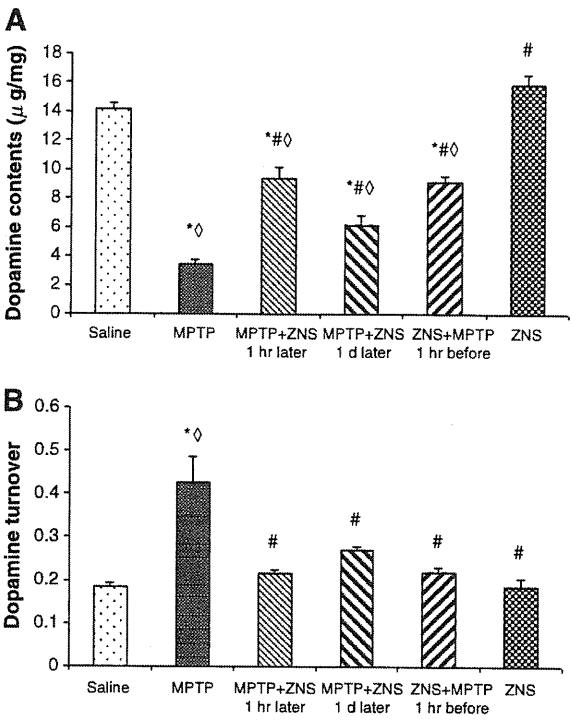

Fig. 8 – Effects of zonisamide administered 9 weeks after MPTP on (A) dopamine contents and (B) dopamine turnover in striatum. Values are presented as the mean ± SEM (n = 6–10 per group). The levels of significance were analyzed by ANOVA, with *post hoc* Tukey test: sequence of groups and symbols for statistical analysis are same with those in Fig. 1.

astrocytes worked to remove dead cells and S100β-positive astrocytes played neurotrophic effects for repairing damaged cells. However, after 3 weeks after MPTP, the activity of S100β-positive astrocytes and GFAP-positive astrocytes returned near to normal control level (see Supplementary Fig. 1).

In conclusion of this study, it may be suggested that zonisamide initiated prolong recovery of dopaminergic cell damage in the MPTP-treated mice by an increase of S100β and, after several weeks, these dopaminergic neurons had an increased capacity for dopamine production. Furthermore, zonisamide increased the TH-contents of dopamine neurons that may assist in the survival of damaged dopamine neurons. Zonisamide might therefore be helpful in suppressing the degeneration of dopamine neurons during the treatment of Parkinson's disease.

4. Experimental procedures

4.1. *Animals*

C57BL/6J male mice with 9–10 weeks of age were used in this study. Five mice per cage were housed in a controlled environment (temperature, 25 ± 1 °C; humidity, 50% ± 5%). A 12 h light/dark cycle was maintained with free access to food and water. The number of animals used for our study is briefly described in Supplementary Table 1. The study was approved by the Committee of Animal Experimentation, Ehime University Graduate School of Medicine.

4.2. *Drugs*

MPTP (Sigma, St. Louis, MO) and zonisamide (Nakarai Tesque, Kyoto, Japan) were purchased and stored at room temperature. MPTP was prepared in a laminar flow cabinet.

4.3. *Drugs administration*

The mice were divided into six groups and all drugs were administered subcutaneously as mentioned briefly in Supplementary Table 2. Three series of experiments were conducted: brains from the mice of series 1, 2, and 3 were dissected out at 1, 3, and 9 weeks, respectively, after the first dose of MPTP.

4.4. *High performance liquid chromatography*

The brain of animals of each group from series 1 (n = 7–9), series 2 (n = 6–10), and series 3 (n = 6–10) assigned for high performance liquid chromatography (HPLC) were dissected out, quickly put on an ice-cold glass plate, and stored at −80 °C until assayed. The contents of dopamine (DA) and its metabolites, dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA), in the brains were measured by HPLC (see Supplementary Text 1). The ratio of (DOPAC + HVA)/DA in the

striatum was calculated to determine dopamine turnover in each animal.

4.5. *Immunohistochemistry*

Brains of animals from series 1 ($n=6$) assigned for immunohistochemistry were dissected on an ice-cold glass plate and fixed with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for 48 h at 4 °C and routinely embedded in paraffin. Paraffin blocks were cut into 7 μm thick section by microtome at the level of substantia nigra. For morphological analysis of the central part of substantia nigra, the sections (−3.52 mm from bregma and 0.28 mm from interaural) (Franklin and Paxinos, 1996) were processed for immunohistochemistry (see Supplementary Text 2). The numbers of TH-positive neurons and GFAP- and S100β-positive astrocytes in substantia nigra were counted manually under "blind conditions" (i.e., observer not aware of group allocation of the sections) by three different observers and averaged. Eighteen large, distinct TH-positive dopamine nucleated neurons from each group were randomly chosen and their cell area was measured using the BZ-Analyzer Program (Keyence, Tokyo, Japan).

4.6. *Statistical analysis*

One-way analysis of variance (ANOVA) was used for data analysis, and multiple comparisons were corrected with Tukey test. Results with P value <0.05 were considered significant. All results are expressed as mean values±standard error of the mean (SEM).

Supplementary materials related to this article can be found online at doi:10.1016/j.brainres.2011.02.017.

Disclosure

The authors have no conflicts to disclose.

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ORIGINAL

View and present status of personnel involved in clinical trials : a survey of participants from the First Symposium of the Shikoku Collaborative Group for Promotion of Clinical Trials

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Abstract : Clinical trials leading to drug approval (registration trials) play a central role in the drug development process. Since the introduction of the Good Clinical Practice (GCP) standard in 1997, the Japanese infrastructure for registration trials has improved. The contribution of support staff, including clinical research coordinators (CRCs), to clinical trials is now widely recognized in Japan. Quality issues and career development for these support staff are being increasingly emphasized. The Shikoku Collaborative Group for Promotion of Clinical Trials was organized in 2008 to address these issues through communication with the personnel involved in clinical trials in regional areas of Japan. To understand the views and present status of personnel involved in clinical trials, we used questionnaires to survey the participants of the First Symposium of the Shikoku Collaborative Group for Promotion of Clinical Trials held in August 2009. Group discussions and special lectures occurred at the symposium. The questionnaire began with questions about basic patient characteristics, followed by practical questions. Of 110 participants, there were 68 respondents (62%), including clinical trial support staff (clinical research coordinators [n=36, 53%], administrative officers [n=9, 13%]), and medical staff [n=23, 34%]). Among the support staff, 36 (80%) had more than 5 years of experience. The most common questionnaire answer selected for participation in the symposium was "willing to contact staff from other medical institutions or organizations" for support staff and "to obtain further knowledge concerning clinical trials" for medical staff. The overall view of the discussion ("Was the discussion satisfactory?") was favorable for 36 (53%) respondents. This survey revealed that the group discussion in the present symposium appears to be valuable for participants, using overall satisfaction as a surrogate. Based on the information obtained in the present study, further development of the clinical trial infrastructure, including training opportunities and career development for support staff, is required. Due to the limitations of this study, further analysis is warranted to determine the optimal strategy for training support staff. J. Med. Invest. 58 : 81-85, February, 2011

Keywords **:** *clinical trial, clinical research coordinator, training, regional area*

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INTRODUCTION

Clinical trials leading to drug approval (registration trials) play a central role in the drug development process, and clinical trials in the general

practice setting are important for providing evidence about the efficacy and safety of different agents in various settings. Since the introduction of the Good Clinical Practice (GCP) standard in 1997 and the plan for the promotion of registration trials by the Ministry of Health, Labor and Welfare and the Ministry of Culture and Science of Japan, the Japanese infrastructure for registration trials has improved. For example, the contribution of clinical research coordinators (CRCs) to clinical trials is now widely recognized. Recently, a marked increase both in the absolute numbers and in the percentage of clinical trial notifications of global clinical trials that include Japanese subjects has been observed (1), and conducting clinical trials has become increasingly complicated in Japan as well as in the global settings (2). Quality issues regarding support staff, in addition to investigators, are now being emphasized to ensure celerity and high quality in clinical trial conduct.

To address clinical trial support staff quality issues, various certification systems have been developed by societies, such as the CRC certification by the Japanese Society for Clinical Pharmacology and Therapeutics, and nationwide opportunities for learning and training have also been developed. In addition, person-to-person communication and discussion of issues, including support staff quality issues, appears to be a reasonable strategy for promoting clinical trials among personnel involved in clinical trials in regional areas. For this purpose, the Shikoku Collaborative Group for Clinical Trials was organized in 2008 by the personnel of departments concerned with promotion of clinical trials in the four university hospitals in the Shikoku area of Japan. Since understanding the view and present status of personnel involved in clinical trials may contribute to future activity of the group, we used questionnaires to survey the participants of the First Symposium of the Shikoku Collaborative Group for Clinical Trials held in August 2009 at Tokushima University Hospital. Herein we present the results of this analysis.

METHODS

The symposium was open to medical staff, contract research organization staff, and site management organization staff, and consisted of two parts. The first part involved group discussions about issues facing clinical trial management, such as basic activity of CRCs, promotion of clinical trial enrollment, multi-national clinical trials, CRC career paths, and the administrative work involved in clinical trials. The participants discussed each of these issues for approximately 1.5 hours in small groups (n=8-12). The second part consisted of special lectures presented by clinical trial experts. After the symposium, a questionnaire was given to each participant. The questionnaire began with questions about basic participant characteristics. This was followed by practical questions about issues such as the main reason for participating in the symposium and views on present training opportunities using a five-point scale (strongly agree, agree, neutral, disagree, and strongly disagree).

We compared the views of clinical trial support staff (CRCs and administrative officers) with those of medical staff, and categorical variables were analyzed using the χ^2 test. P values < 0.05 were considered to be statistically significant. All P values were based on two-sided tests.

RESULTS

Respondent characteristics

Of 110 symposium participants, 68 questionnaire respondents (62%) were included in this analysis. These respondents included CRCs (n=36, 53%), administrative officers (n=9, 13%), and medical staff (physicians, n=3, 4%; pharmacists, n=16, 24%; nurses, n=3, 4%; and a clinical laboratory technician, n=1, 2%).

Among the clinical trial support staff (CRCs and administrative officers, n=45), 36 (80%) had more than 5 years of experience, whereas 9 (20%) had less than 1 year of experience. With respect to previous experience with small-group clinical trial training, 17 respondents had never participated in small-group training (38%), 9 had participated in small-group training one time (20%), 9 had participated in it two times (20%), and 3 had participated in it more than two times (7%); 7 participants provided no answer.

Certification of CRC respondents

Only 7 (19%) of the responding CRCs (n=36) had already acquired certification by the Japanese Society for Clinical Pharmacology and Therapeutics, while 5 expressed their willingness for future acquisition of this certification.

Main reason for participating in the symposium

Select answers to the survey questions are shown in Table 1. The main reasons for participation were as follows : willing to attend the first such symposium in the Shikoku district (n=17, 25%), willing to contact staff from other medical institutions or organizations (n=16, 24%), to obtain further knowledge concerning clinical trials (n=30, 44%), and other (n=5, 7%). The "willing to contact staff from other medical institutions or organizations" answer was observed at a significantly higher frequency (P=0.008) in support staff (n=15, 33%) compared to medical staff (n=1, 4.3%), whereas the "to obtain further knowledge concerning clinical trials" response was observed at a significantly higher frequency (P=0.012) in medical staff (n=15, 65%) compared to support staff (n=15, 33%).

View of the training method itself

As noted above, each participant discussed each issue for about 1.5 hours in small groups (n=8-2). A considerable number of respondents (27 [40%]) wanted to participate in discussions in groups consisting of fewer individuals (<8), and 43 (63%) wished to hold the discussions for a longer period of time. The discussions were open to medical staff, contract research organization staff, and site management organization staff. The majority of respondents (44, 65%) agreed that participation from sponsors, such as pharmaceutical companies, would be welcomed.

The overall views of the discussion ("Was the discussion satisfactory?") were as follows : strongly agree (12 [18%]), agree (24 [35%]), neutral (12 [18%]), disagree (3 [4%]), and strongly disagree (1 [2%]) ; 16 (24%) respondents provided no answer.

DISCUSSION

Before the introduction of the CRC concept, investigators participating in Japanese registration trials performed virtually all tasks related to the trial, from patient care to administrative work, throughout the course of the study (3). The importance of the contribution of CRCs to clinical trials is now widely recognized, even in Japan. In our previous study conducted at the Tokushima University Hospital, over 80% of the doctors requested CRC support throughout the registration trial (4). In addition, we found that physicians who could recruit participants into a trial considered the presence of a support system with CRCs as the reason to participate in the trial (5).

In the global setting, Gets *et al*. (2) at the Tufts Center for the Study of Drug Development analyzed data on protocols and study conduct performance in clinical trials conducted between 1999 and 2005. These investigators reported that the number of unique procedures and the frequency of procedures per protocol had increased, and investigative site work burden to administer each protocol increased at an even faster rate. Additionally, study conduct performance-that is, cycle time and patient recruitment and retention rates-worsened. In Japan, a marked increase both in the absolute numbers and in the percentage of clinical trial notifications of global clinical trials that include Japanese subjects has occurred in recent years (1). While cancer and cardiovascular disease have been the major target diseases of these trials, the range has recently expanded to include other diseases.

In consideration of these circumstances, improving the skills of clinical trial support staff, such as CRCs and administrative staff, in addition to those

Table 1 Main reason for participating in the symposium

	Total (n=68)	Support staff (n=45)	Medical staff (n=23)
Willing to attend the first such symposium in the Shikoku district	17 (25.0%)	13 (28.9%)	4 (17.4%)
Willing to contact staff from other medical institutions or organizations	16 (23.5%)	15 (33.3%)*	1 (4.3%)
To obtain further knowledge concerning clinical trials	30 (44.1%)	15 (33.3%)	15 (65.2%)**
Other	5 (7.4%)	2 (4.4%)	3 (13.0%)

* Significantly different (P=0.008) than medical staff
** Significantly different (P=0.012) than support staff

of the investigators, is now being emphasized at each medical institution to ensure efficient progress and improved quality of clinical trials. Moreover, support staff-specific issues, such as CRC career development, are now also identified as being important. The Shikoku Collaborative Group for Clinical Trials was organized in 2008 to fulfill these demands in the Shikoku area in Japan, and we conducted the present analysis to promote activity of the group by understanding the views and present status of the personnel involved in clinical trials.

To improve the skills of CRCs in Japan, obtaining and maintaining certification by the Japanese Society for Clinical Pharmacology and Therapeutics would appear to be desirable. However, in the present survey, few CRCs had already acquired or expressed their willingness for future acquisition of this certification. The possibility that regional differences, such as a relatively smaller number of clinical trials conducted in regional areas compared to urban areas, could result in the "less" requirement for skilled CRCs should be considered. In the group discussions about CRC career development, many CRCs insisted that there is little incentive to becoming a CRC and that no organized career path for CRCs exists (data not shown). Development of a clear CRC career path is an important issue for this collaborative group to resolve.

Regarding the reason for participating in the present symposium, more supportive staff than medical staff showed their willingness to contact staff from other medical institutions or organizations. From the perspective of the CRC, medical staffs, such as ward-based clinical nurses work as a nursing team. On the other hand, CRCs must be able to competently perform their research roles and must adapt to working alone and working with a variety of clinical professionals. CRCs often feel insecure and feel that they are perceived as a minority group, and that their complaints cannot be accepted by their colleagues who lack understanding and insight into the research process (6). Feelings of isolation and tension throughout clinical trials exist, even after CRCs have gained skills and confidence in their roles (7). Although it is now widely accepted that CRCs play important roles in ensuring the quality of clinical trials while lessening the workload of physicians, networking of CRCs, such as in the present collaborative group, could be beneficial for the support staff, including CRCs, to lessen their feelings of isolation, and may contribute to more efficient job performance.

Various methods exist for training medical staff, including CRCs. Compared to self-study, investigator meetings, and lectures, small-group discussions have unique value in their interactivity and immediate feedback. In the present analysis, discussions of approximately 1.5 hours in length in small groups (n=8-12) appeared to be unsatisfactory to the participants. Longer discussion periods with participation of various professionals, including monitors and audit-related individuals from sponsors, such as pharmaceutical companies, should be considered for future small-group discussions. Taekman *et al*. (8) published a preliminary report on the use of high-fidelity simulation in the training of study coordinators conducting a clinical research protocol. Improvement of training methodology and evaluation of the contribution of training to clinical trial quality should be considered as a future goal of this collaborative group.

The present study evaluated only a small number of professionals involved in clinical trials. Nevertheless, the group discussion format used in the present symposium appears to be of value to participants when overall satisfaction is used as a surrogate. It is necessary to develop the proper infrastructure, including training opportunities, in the Shikoku area, based on the information obtained in the present study. Because of the study limitations, further study is warranted to determine the generalizability of the present findings to other Japanese regional areas.

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ORIGINAL RESEARCH

Comparison of peer-led versus professional-led training in basic life support for medical students

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Background: The effect of peer-led training in basic life support (BLS) in the education of medical students has not been assessed.

Subjects and methods: This study was a randomized controlled trial with a blinded outcome assessor. A total of 74 fourth-year medical students at Ehime University School of Medicine, Japan were randomly assigned to BLS training conducted by either a senior medical student (peer-led group) or a health professional (professional-led group). The primary outcome measure was the percentage of chest compressions with adequate depth (38–51 mm) by means of a training mannequin evaluated 20 weeks after BLS training. Secondary outcome measures were compression depth, compression rate, proportion of participants who could ensure adequate compression depth (38–51 mm) and adequate compression rate (90–110/minute), and retention of BLS knowledge as assessed by 22-point questionnaire.

Results: Percentage chest compressions with adequate depth (mean ± SD) was 54.5% ± 31.8% in the peer-led group and 52.4% ± 35.6% in the professional-led group. The 95% confidence interval (CI) of difference of the means was –18.7% to 22.8%. The proportion of participants who could ensure an adequate mean compression rate was 17/23 (73.9%) in the peer-led group but only 8/22 (36.4%) in the professional-led group ($P = 0.011$). On the 22-point questionnaire administered 20 weeks after training, the peer-led group scored 17.2 ± 2.3 whereas the professional-led group scored 17.8 ± 2.0. The 95% CI of difference of the means was –1.72 to 0.57.

Conclusion: Peer-led training in BLS by medical students is feasible and as effective as health professional-led training.

Keywords: basic life support, education, training, randomized controlled trial

Introduction

Providing basic life support (BLS) is an essential skill for the survival of a patient after cardiac arrest. Bystander cardiopulmonary resuscitation, one of the important skills in BLS, is associated with increased chances of survival with a twofold increase in survival rate.[1] With more people trained in BLS, more lives can potentially be saved; however, the high cost in terms of time, money, and opportunity of traditional training programs limits the number of citizens trained to perform BLS.

Employing medical students as instructors could help increase the number of people with BLS skills. Several studies showed that medical students could successfully teach BLS to lay people.[2–4] Their teaching skills and the training outcome, however, were poorly evaluated.

Peer teaching and learning[5] is a potentially effective method in medical education. Peer-led training in BLS by pairing senior and junior healthcare students can provide BLS skills of equally good quality as that provided by professional-led training.[6,7] The Japanese system of medical education, however, is focused on education in a paternalistic manner.[8] Although the concept of peer-led training in BLS is not new,[9] arguments against peer teaching and learning by medical students are still common in Japan.

Therefore we conducted a study to compare peer-led training versus health professional-led training in a randomized controlled trial. The primary aim of our study was to determine whether medical students could offer a level of training comparable with that provided by experts.

Subjects and methods

Study design and participants

From April to May 2009, we conducted a randomized controlled trial approved by the Institutional Review Board of Ehime University School of Medicine. Figure 1 shows the flow of participants in the trial. We recruited fourth-year medical students attending an objective structured clinical examination (OSCE) curriculum at Ehime University School of Medicine. None of the participants had prior BLS training. After the study aims were explained, the participants, who provided written informed consent, were allocated according to a Latin square design to either of the following two groups: one group instructed by senior medical students (peer-led group) and the other group instructed by healthcare professionals (professional-led group).

Intervention (training)

Two-hour BLS training was conducted according to American Heart Association (AHA) guidelines.[10] One instructor taught three or four participants with a training mannequin and a training video (BLS for Healthcare Providers Video by AHA). The peer instructors were nine volunteers (five fifth-year and four sixth-year students) recruited from a student-run group interested in BLS. All had undergone BLS training in their fourth year and passed the OSCE. Six (three fifth-year students and three sixth-year students) had completed the Immediate Cardiac Life Support (ICLS) course sponsored by Japanese Association for Acute Medicine (http://www.icls-web.com/index.html). The professional instructors were 7 emergency medical technicians, 2 anesthesiologists, and 2 nurses. The emergency medical technicians and nurses had completed the ICLS course; the anesthesiologists had completed the BLS provider course.

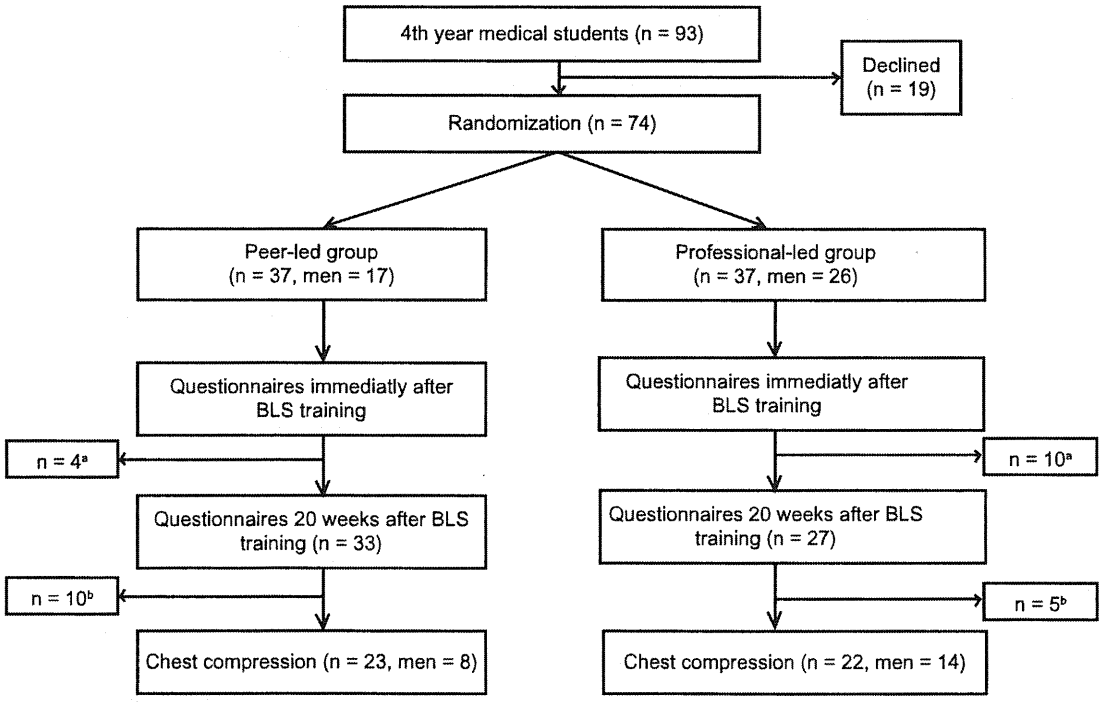

Figure 1 Flow of participants through trial.
Notes: [a]Did not return for evaluation 20 weeks after basic life support (BLS) training. [b]Declined evaluation. The data are presented as means ± standard deviation.

Outcome measurements

We assessed BLS skills with the Laerdal Skill Reporter Resusci Anne and Acquisition™, a full-body recording mannequin with a skill-reporting system.[11] At 20 weeks after BLS training, participants were asked to perform continuous chest compressions on a mannequin for 3 minutes. For each resuscitation attempt, the rate and depth of compressions were recorded.

The participants' retention of knowledge was evaluated immediately and 20 weeks after BLS training by 22-point questionnaire.[4] Question 22 (chest compression rate) was adjusted according to the International Liaison Committee on Resuscitation (ILCOR) guidelines of 2005.[10] Question 7 was altered according to the relevant ambulance call number in Japan.

The primary outcome measure was percentage chest compressions with adequate depth (38–51 mm). Secondary outcome measures were compression depth, compression rate, proportion of participants who could ensure an adequate mean compression depth (38–51 mm) and adequate mean compression rate (90–110/minute),[11] and the score on the 22-point questionnaire. The adequate depth and rate of chest compressions were set according to a previous study[11] and 2005 AHA guidelines.

Blinding

Clearly, it was not possible to blind the participants and instructors to their allocated groups. However, the computer screen of the skill-reporting system was not visible to the participants and no feedback from the screen was given.[11] The questionnaires were scored by an investigator who was blinded to the status of the participants.

Statistical analysis

We did not perform a power calculation to determine the sample size to detect the differences between the two groups because of the limitations of our setting. Since the number of fourth-year medical students attending an OSCE within their regular medical training was 93, we were limited to recruitment of a maximum of that number. Therefore to compare the outcome measures between the groups we calculated the 95% confidence intervals (CIs) for the difference between the two means instead of detecting a significant difference. We used the chi-square test to compare the proportion of participants who could ensure adequate compression rates or depths between the two groups.

Results

In total, 93 fourth-year students were recruited. The baseline characteristics of the participants were similar between the two groups (Table 1). Figure 1 shows the flow of participants through the trial. The professional-led group consisted of more men. A total of 14 participants (four in the peer-led group and ten in the professional-led group) did not return for the 20-week follow-up after training. Moreover, 15 participants (ten in the peer-led group and five in the professional-led group) declined the evaluation of chest compressions. In total, of the 74 participants who underwent BLS training, 45 (23 in the peer-led group and 22 in the professional-led group) participated in the evaluation of chest compressions.

Table 2 summarizes the outcome measures. The primary outcome (percentage chest compressions with adequate depth) was similar between the two groups, with a 95% CI of the difference of the means –18.7% to 22.8%. One of the secondary outcome measures, proportion of participants who could ensure adequate compression rate, was 17/23 (73.9%) in the peer-led group but only 8/22 (36.4%) in the professional-led group ($P = 0.011$). The questionnaire scores were similar between the two groups.

Discussion

This trial showed that peer-led training in BLS by medical students is feasible and as effective as health professional-led training. The primary outcome measure, percentge chest compressions with adequate depth, was similar between the two groups, although the broad 95% CI of the difference between the means of the measure was inconclusive. Other outcome measures showed that the peer-led group performed as well as or better than the professional-led group. The proportion of participants who could ensure adequate compression rate was significantly higher in the peer-led group than in the professional-led group. On the questionnaire score, the narrow 95% CIs of the difference in the means between the two groups both immediately and at 20 weeks after BLS training also suggest that training provided by the peer medical students was not inferior to that by the professionals.

Table 1 Demographic characteristics of student participants

	Peer-led group	Professional-led group
Age (years)	23.6 ± 1.5	24.0 ± 1.3
Height (cm)	164.8 ± 7.5	167.3 ± 7.5
Weight (kg)	57.4 ± 12.9	58.0 ± 9.0

Note: Values are mean ± standard deviation.

Table 2 Comparison of outcome measures between peer-led group and professional-led group

Chest compression	Peer-led group	Professional-led group	Difference of means (95% CI) or *P* value*
Compression depth (mm)	43.5 ± 7.6	42.8 ± 9.5	0.75 (–4.5 to 6.0)
Percent compression with adequate depth (38–51 mm) (%)	54.5 ± 31.9	52.5 ± 35.6	2.0 (–18.7 to 22.8)
Proportion of participants with adequate compression depth, n (%)	16/23 (69.6)	12/22 (54.5)	$P = 0.299$
Compression rate (per minute)	104.3 ± 9.1	109.6 ± 10.6	–5.3 (–14.4 to 3.7)
Proportion of participants with adequate compression rate (90–110/minute), n (%)	17/23 (73.9)	8/22 (36.4)	$P = 0.011$
Questionnaire scores			
Immediately after BLS training	18.2 ± 1.4	18.0 ± 1.7	0.27 (–0.45 to 0.99)
At 20 weeks after BLS training	17.2 ± 2.3	17.8 ± 2.0	–0.57 (–1.72 to 0.57)

Notes: Values are mean ± SD unless otherwise specified; *chi-square test.
Abbreviation: BLS, basic life support.

Medical students have been reportedly successfully involved in teaching BLS in various settings.[2,4,12–14] In these studies, however, the teaching skills of medical students were not compared with those of experts. Additionally, evaluation of the quality of educational training is often difficult. Significance of randomized controlled trials, which provide strong evidence, remains to be established in medical education.[5] Previous studies,[6,7] however, adopted this design to compare peer-led with expert-led resuscitation. Perkins et al[6] showed that undergraduate medical, dental, nursing, and physiotherapy students taught by their peers were more successful in an examination in BLS than those taught by clinical staff. In another example, a randomized controlled trial[7] showed that advanced cardiac resuscitation can be safely and effectively taught to medical students by their peers. To judge the quality of educational interventions, these studies use evaluation data from students, eg, the exam pass rate, rather than outcomes. In the present study, we used parameters in chest compressions as feasible outcome measures for comparison, as well as questionnaire scores.

In Japan, individuals who are familiar with the traditional approach towards education, which stresses didactic lectures, book-learning, and memorization,[8] are concerned about the quality of peer-led training by medical students. Contrarily, this study provides evidence supportive of adequate teaching skills of students even in the traditional setting of medical education. In Japan, about 30 medical student-run organizations teach BLS. More than 800 medical students annually participate in this activity.[15]

We acknowledge, however, that the study has certain limitations. First, owing to the limited number of medical students, we could not perform a power calculation to determine the sample size to detect differences between the two groups or to demonstrate noninferiority. Second, 29 people declined participation after randomization, which may have led to bias. Third, the instructors of the peer-led group were recruited from a student-led interest group in BLS. This may have caused an overestimation of the effect of the training.

Conclusion

Peer-led training in BLS by medical students is feasible and as effective as health professional-led training, although a much larger, randomized controlled trial is needed to provide stronger evidence. Our findings suggest that medical students are valuable resources to teach BLS skills.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work. Trial registration UMIN-Clinical Trials Registry UMIN000001533.

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INTERNAL MEDICINE

□ CASE REPORT □

Three Spinocerebellar Ataxia Type 2 Siblings with Ataxia, Parkinsonism, and Motor Neuronopathy

Noriko Nishikawa[1], Masahiro Nagai[1], Tomoaki Tsujii[1], Nachi Tanabe[1], Hiroshi Takashima[2] and Masahiro Nomoto[1]

Abstract

Spinocerebellar ataxia type 2 (SCA2) represents a family of dominant neurodegenerative disorders that results from CAG expansion repeat mutations. The phenotype consists of some common features, most notably progressive ataxia. We describe three siblings with SCA2, manifesting parkinsonism and ataxia in the first sibling, juvenile parkinsonism in the second and motor neuronopathy in the third. Genetic examination revealed expansion to 42, 43, and 42 CAG repeats. There was no relationship between the number of repeats and phenotype. The SCA2 gene should be studied in families with heterogeneous neurodegenerative disorders, including motor neuron disease.

Key words: SCA2, motor neuron disease, parkinsonism, ataxia, neurodegenerative disorders

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Introduction

Autosomal dominant cerebellar ataxia type 2 is caused by CAG expansion in the cording region of the ataxin 2 gene on chromosome 12q23-24.1. The normal range of CAG repeats usually extends from 14 to 32 repeats, while it ranges from 35 to 50 or more in affected persons (1, 2). The clinical hallmark of spinocerebellar ataxia type 2 (SCA2) with juvenile onset is cerebellar gait and limb ataxia associated with slow eye movements and hyporeflexia. However, it has been shown recently that the phenotype of SCA2 is wider than previously believed. Patients may present with either a typical L-dopa-responsive parkinsonism or an atypical parkinsonism including signs of ataxia (3). There may be considerable intra- and interfamilial variation of clinical signs (4). We describe three siblings with SCA2 CAG expansion, one sibling presented with parkinsonism and ataxia, the second one with juvenile parkinsonism, and the third one with motor neuronopathy. We investigated the relationship between phenotype and genotype.

Case Report

Three siblings were examined after obtaining permission to use their photographs and informed consent was obtained to take blood sampling for genetic study. Genomic DNAs were isolated from peripheral blood lymphocytes using the DNA Extractor WB kit (Wako, Japan). The regions containing the SCA2 CAG repeats were PCR-amplified using previously described gene-specific primers (5′-CCCTCACCAT GTCGCTGAAGC-3′ and 5′-3′) (5). The number of the repeats in the fluorescent-labeled PCR products was estimated by Gene Scan analysis using an ABI PRISM 310 automated DNA sequencer (Applied Biosystems, Foster City CA USA), then, determined through the PCR products sequencing on an ABI PRISM 310 Genetic Analyzer using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

The proband (case 1: III-13) is a 59-year-old man. He had been well until 42 years of age, when he noticed gait difficulties. At 45 years, he was diagnosed with PD. His mother (II-8), sister (case 2: III-14), uncles (II-4,5) and aunts (II-2,7) of the mother's side had been treated under the diagno-

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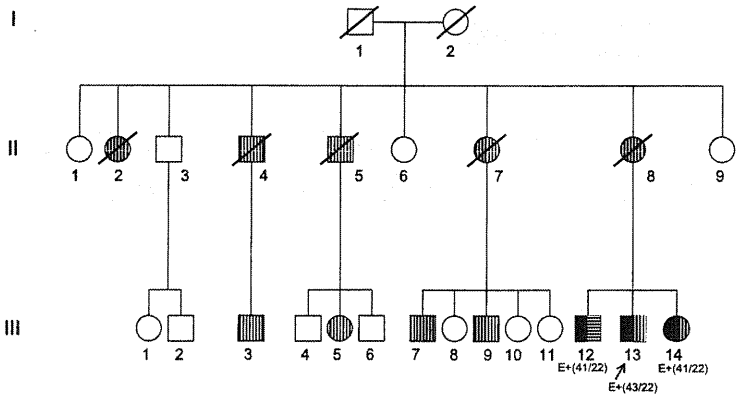


Figure 1. **Pedigree of the three siblings. E+(CAG repeat numbers) indicate positive evaluation with the number of CAG repeats in the expanded and the normal alleles.**
Indicates SCA2
Indicates parkinsonism
Indicates motor neuronopathy

sis of Parkinson's disease. The family history suggested they were affected by hereditary parkinsonism with autosomal dominance (Fig. 1). He showed mild rigidity and bradykinesia. No limb or gait ataxia was noted. Levodopa/carbidopa was prescribed with marked benefit. The medication allowed him to perform activities of daily living. He kept his job as a local government employee until the age of 56. However, his symptoms progressed gradually. At the age of 57, he developed dysarthria and trunkal ataxia. Brain magnetic resonance imaging (MRI) study revealed brainstem and cerebellar atrophy (Fig. 2a).

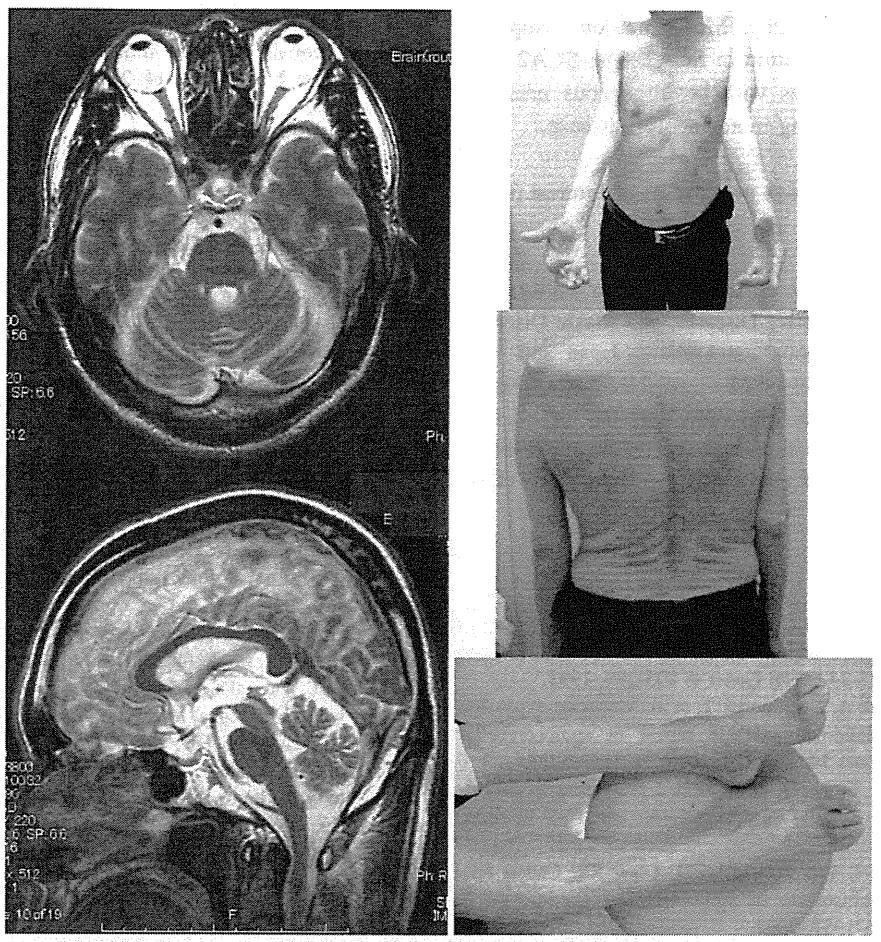
Case 2 (III-14) is the younger 58-year-old female sibling of the proband (III-13). At age 39, she developed resting tremor and rigidity, and bradykinesia. She was diagnosed with juvenile PD. She responded to levodopa very well, keeping her job perfectly for 15 years as an office worker for an insurance company. She sometimes showed mild trunkal and leg dyskinesia during "ON" time with levodopa treatment. She did not show ataxia, abnormal eye movements, pyramidal signs, nor significant dysautonomia except for constipation. Brain MRI revealed no abnormalities. The ratio of myocardial ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphic uptake in regions of interest in the heart to that in the mediastinum (H/M ratio) was reduced (early 1.26, delay 1.09) (6). Her phenotype was indistinguishable from idiopathic PD.

Case 3 (III-12) is the elder 64-year-old male sibling of the proband (III-13). Marginal muscle weakness and atrophy in the upper limbs was noted at 14 years of age. The muscle weakness was slowly progressive. However, he could manage everything in his life as a business person up to the age of 60 years. He did not show signs or findings suggestive of poliomyelitis or exposure to toxic substances that cause muscle weakness. Neurological examination disclosed muscle atrophy in the neck, shoulder girdle, and limbs (Fig. 2b). He did not show ataxia, parkinsonism, or pyramidal signs. Brain MRI revealed no abnormalities. Electrophysiological findings were consistent with a chronic neurogenic pattern. Nerve conduction study was normal with no evidence of conduction block. Compound muscle action potential was low, which was consistent with muscle atrophy.

The siblings had a normal allele with 22 repeats that sequencing showed glutamines were encoded by $(CAG)_8$-$(CAA)(CAG)_4(CAA)(CAG)_8$. The expanded allele of case 1 had 43 glutamine repeats encoded by $(CAG)_{34}(CAA)(CAG)_8$. Case 2 and case 3 had 42 glutamine repeats encoded by $(CAG)_{33}(CAA)(CAG)_8$ (Fig. 3). The number of repeats was increased by two in case 1 compared to case 2 and case 3, and there were no differences between case 2 and case 3 in the genetic investigation.

Discussion

This family had been noticed as being affected by hereditary PD with autosomal dominance. The proband case showed ataxia 12 years after the development of parkinsonism and was shown to have SCA2 mutation on gene analysis. Case 2 showed parkinsonism but did not develop ataxia until 19 years after PD onset, when she showed balance disturbance and CT scan confirmed mild cerebellar atrophy. MRI study did not show cerebellar atrophy. MIBG study revealed a decreased H/M ratio which is compatible with parkinsonism, while the other 2 cases (1 and 3) showed H/M ratio values of 2.1 and 1.9, respectively, which are normal. Case 3 developed bilateral muscular atrophy of the arms. Cases with SCA2 exhibiting muscular atrophy and cerebellar ataxia or rigidity have been previously reported (7, 8). Case 3 started to develop muscle weakness and atrophy of the arms at 14 years of age, which worsened very slowly. He was not affected by poliomyelitis, with his serum titer being lower than the detectable limit. Nerve conduction velocity was normal, but there was a suggestion of spinal cord motor neuron degeneration. The CAG repeat expansion in SCA2 gene was detected in case 3. Pathological



a b

Figure 2. **Brain MRI of case 1 (III-13) (a) and pictures of case 3 (III-12) (b).**

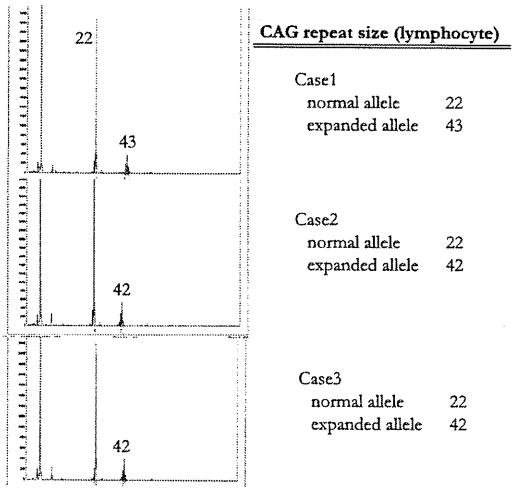


Figure 3. **Fragment analysis of SCA2 gene in the three siblings.**

study has previously revealed cases of SCA2 showing motor neuron degeneration (9). Case 3 (III-12) may be a phenotype of SCA2 and should be followed up for the possible development of ataxia or parkinsonism. Pathological study will be recommended for the motor neuronopathy in the future.

Patients with parkinsonism-predominant SCA2 without ataxia have been recently described to respond dramatically to levodopa therapy. These cases are reported in Asians, but rarely in Caucasians. The present cases are compatible with these reports of PD (3, 4, 7). CAG repeats which were in the low expansion range and interrupted by CAA were associated with SCA2-related parkinsonism (10, 11). Another finding about SCA disease is the large variation of the phenotype. SCA2 has been classified by OPCA, and its phenotype seems to be related to the length of CAG repeats (12, 13). However there was no difference in the length on CAG repeats or the gene sequence in our siblings. Their phenotype varied and they were diagnosed as different disorders clinically. There may be other factors apart from the length or sequence of CAG repeats that determine SCA2 phenotype. CAG repeat size can be different between tissues such as cerebellum, pons, or spinal cord (14). Genotypic examination for SCA2 should be considered more widely because of the varied phenotype (15).

In conclusion, we have described three siblings with SCA 2, who developed juvenile parkinsonism, parkinsonism/

ataxia, and motor neuronopathy. Motor neuron symptoms and signs may be a manifestation in SCA2. The SCA2 gene should be studied in families with heterogeneous neurodegenerative disorders, including motor neuron disease.

The authors state that they have no Conflict of Interest (COI).

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A cytokine mixture of GM-CSF and IL-3 that induces a neuroprotective phenotype of microglia leading to amelioration of (6-OHDA)-induced Parkinsonism of rats

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Abstract

Dopamine (DA) agonists are widely used as primary treatments for Parkinson's disease. However, they do not prevent progressive degeneration of dopaminergic neurons, the central pathology of the disease. In this study, we found that subcutaneous injection of a cytokine mixture containing granulocyte macrophage colony-stimulating factor and interleukin-3 (IL-3) markedly suppressed dopaminergic neurodegeneration in 6-hydroxydopamine-lesioned rats, an animal model of Parkinson's disease. The cytokine mixture suppressed the decrease of DA content in the striatum, and ameliorated motor function in the lesioned rats. In response to the cytokine injection, dopaminergic neurons in the substantia nigra pars compacta increased expression of the antiapoptotic protein Bcl-xL. Microglial activation in the pars compacta was evident in both the saline- and cytokine-injected rats. However, the cytokine mixture suppressed expression of the proinflammatory cytokines IL-1β and tumor necrosis factors α, and upregulated the neuroprotective factors insulin-like growth factor-1 and hepatocyte growth factor. Similar responses were observed in cultured microglia. Detailed morphometric analyses revealed that NG2 proteoglycan-expressing glial cells increased in the cytokine-injected rats, while astrocytic activation with increased expression of antioxidative factors was evident only in the saline-injected rats. Thus, the present findings show that the cytokine mixture was markedly effective in suppressing neurodegeneration. Its neuroprotective effects may be mediated by increased expression of Bcl-xL in dopaminergic neurons, and the activation of beneficial actions of microglia that promote neuronal survival. Furthermore, this cytokine mixture may have indirect actions on NG2 proteoglycan-expressing glia, whose role may be implicated in neuronal survival.

Introduction

Parkinson's disease (PD) is a very common neurodegenerative disorder, which is characterized by resting tremor, impaired balance and coordination, bradykinesia, and rigidity. The main pathology of PD is progressive degeneration of dopaminergic (DArgic) neurons in the substantia nigra, pars compacta (SNpc). DArgic neurodegeneration results in decreased dopamine (DA) content in the striatum, which is the major cause of motor disability in PD. Therefore, current PD treatments are mostly focused on replenishing DArgic activity in the striatum by administering L-DOPA or other DA agonists to PD patients. However, this type of therapy does not suppress the DArgic neurodegeneration. Therefore, novel treatments are being sought that mitigate neuronal loss in PD (Yacoubian and Standaert 2009).

In addition to neurodegeneration, glial cell activation has been shown as a pathologic feature of PD (Mosley et al. 2006; McGeer and McGeer 2008; Tansey and Goldberg 2010). Therefore, it is speculated that treatments that affect glial