prepared for physical measurement. The physical situation and the present social conditions were described by the SMON patients. Also, a neurological medical examination was conducted.

Results

Thirty people received a medical examination in 2013. They comprised 10 men and 20 women. The average age was 80 years old. The average age at which the disease was contracted was 43 years. The mass checkup covered 21 people. Seven people had a medical examination during an at-home visit. The testees in the Tokushima National Hospital outpatient department numbered two people. The hospitalized patients were alone.

Time of contraction of disease. As shown in Table 1, the age of the patients who had a medical checkup at home was the highest. The age of the patients who had a medical checkup in a group was the second highest. The patients who had outpatient consultations were the youngest. The Barthel Index (73 points) of the patients who had a medical checkup at home was the lowest. Most of the patients who received home care had family medicine. Frequent complications included cataract, hypertension, and arthropathy. The number of patients with a BMI (Body Mass Index) of 25 or more was six this year. Many patients were aware of forgetfulness but in four patients this was complicated by obvious dementia. There were seven elderly people older than 90 years. There were two patients with early onset (onset at 18 years old). Two women patients were 61 years old. One had a part-time job; the other was uneasy about single life in the future.

Discussion

Forty-five years have passed since the sale of the chinoform agent was halted in (1970) in 1970 [1]. As a result, it is over 41 years since SMON patients began to contract the disease. The average disease contraction time of SMON patients in Tokushima prefecture is 43 years. The average age of the testees was 78.

The number of patients in 1972 when a meeting (patients association) of the Tokushima SMON was organized was 155. The medical examination results that we examined corresponded to the national tendency of the average year. Most patients had family medicine. Even if the patients were living alone, a nearby doctor could be contacted in an emergency. Seven patients were over 90 years old. One was over 100 years old. Three people used nursing care insurance. Furthermore, they received close support from family members. There were two women with young onset (18 years old). The Barthel Index scores for them were 95 and 100 points. The degree of their disorder was very mild. As well as support in terms of food, clothing and shelter, mental support seemed to be needed. The weathering measures of the SMON are performed as activity such as "gathering workshops of the SMON" positively in this study squad. The number of medical examination testees of the aging is shown in Table 1. A mass checkup in the Tokushima public health center began in 1990. More than forty people participated constantly from 1999. In 2011, the number of the people having an examination decreased. This may be associated with a decrease in the number of testees to have changed a place in a medical examination this year. However, a decrease in the number of testees due to aging will be a main factor. The number of medical examinations conducted at home should be increased.

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Table 1. Patients with SMON that received a medical examination

	Patients			Mean age	Barthel Index
	Men	Women	Total		
Mass checkup	9	12	21	79	84.5
Checkup at home	1	6	7	87	31.4
Outpatient department	0	2	2	64	97
Hospitalization	0	0	0	-	-

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Superoxide dismutase as a target of clioquinol-induced neurotoxicity

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ABSTRACT

Subacute myelo-optico-neuropathy (SMON) is a progressive neurological disorder affecting the spinal cord, peripheral nerves and optic nerves. Although it has been assumed that SMON was caused by intoxication of clioquinol, the mechanism underlying clioquinol-induced neurotoxicity is not fully understood. This study aimed to clarify the relevance of oxidative stress to clioquinol-induced neurotoxicity and the cause of the enhanced oxidative stress. Clioquinol induced cell death in human-derived neuroblastoma cell line, SH-SY5Y, in a dose-dependent manner. This process was accompanied by activation of caspase-3 and enhanced production of reactive oxygen species (ROS). We examined whether clioquinol inhibited the activity of superoxide dismutase-1 (SOD1), based on its metal chelating properties. Clioquinol inhibited activities of purified SOD1 in a dose-dependent manner. Cytosolic SOD activities were also inhibited in SH-SY5Y cells treated with clioquinol. Finally, addition of exogenous SOD1 to the culture significantly reduced enhanced ROS production and cell death induced by clioquinol in SH-SY5Y cells. These findings suggested that enhanced oxidative stress caused by inhibition of SOD1 undelay clioquinol-induced neurotoxicity and was relevant to the pathogenesis of SMON.

1. Introduction

Subacute myelo-optico-neuropathy (SMON) is a neurological disorder that develops with acute abdominal symptoms, such as abdominal pain and diarrhea, followed by subacute ascending dysesthesia, paresthesia, and muscle weakness of bilateral lower extremities [1–5]. Bilateral visual impairment may occur in severe cases. The syndrome caught attention of physicians particularly in Japan, where it was more prevalent since the middle of 1950's, compared with other areas of the world. According to the 1972 national survey, some 11,000 individuals had been affected by SMON in Japan [2]. The hypothesis that SMON might have been caused by clioquinol, which was popular as an oral drug for amoebic and bacterial dysentery at that time, rose from the discovery of the green hairy tongue in SMON patients. It was confirmed by a remarkable decrease of new SMON cases after the ban on its clinical use in 1970 in Japan [5]. The high prevalence of SMON observed in Japan was attributed to longer-term use of higher doses of clioquinol.

Clioquinol is a derivative of 8-hydroxyquinolone with high lipophilicity and an ability to chelate bivalent metals, particularly copper and zinc. SMON-like pathology was reproduced in experimental animals upon repeated oral administration of clioquinol, and induction of apoptosis in neuronal cells by clioquinol was demonstrated in in vitro cell cultures of neuronal cell lines as well as organotypic cultures of neural tissues [4,6–9]. However, most of studies on SMON are not satisfactory for the current demand of molecular pathobiology, since they were performed in the old times.

Recent efforts to develop a new therapy for Alzheimer disease (AD) have shed new light on a beneficial aspect of clioquinol in clinical use [10,11]. Clioquinol induced a rapid decrease in β-amyloid deposition in a mouse model of AD, and results of a phase II clinical trial of clioquinol in AD patients were promising. Now, clioquinol has been considered as having therapeutic potential on neurodegenerative disorders, not only AD but also Huntington disease and Parkinson's disease [12,13]. To elucidate the mechanism of clioquinol-induced neurotoxicity is becoming more essential beyond the scope of the SMON research.

In this study, we report the involvement of reactive oxygen species (ROS) in clioquinol-induced apoptosis in neuronal cells. We demonstrate that clioquinol inhibits superoxide dismutase-1 (SOD1) activity and enhances ROS production. Furthermore, exogenous SOD1 attenuates ROS production as well as cell death induced by clioquinol.

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2. Materials and methods

2.1. Materials

Clioquinol was purchased from Calbiochem (Billerica, MA). Clioquinol was dissolved in DMSO, to make 10 mmol/L stock solution. The stock solution was further diluted in DMSO before being added to culture medium, so that the concentration of DMSO in clioquinol-containing medium in this study was kept at 1%. Diethyldithiocarbamate (DDC) and SOD1 purified from bovine erythrocytes were from Wako (Osaka, Japan) and Sigma–Aldrich (St. Louis, MO), respectively.

2.2. Cell culture and cell viability assay

SH-SY5Y human neuroblastoma cells were purchased from the American Type Culture Collection (Manassas, VA). Cells were grown in Dublbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS (GIBCO, Carlsbad, CA) and maintained at 37 °C in an environment containing 5% of CO_2.

Cell viability was analyzed using CellQuanti-Blue™ Cell Viability Assay Kit (BioAssay Systems, Hayward, CA). 10,000 SH-SY5Y cells were plated in each well of a 96-well culture plate with 100 μl of culture medium containing clioquinol or DDC and cultured for 24 h. 10 μl of CellQuanti-Blue™ Reagent was added for the last four hours. Fluorescent intensity was measured for each well at 535/595 nm (excitation/emission), using an Infinite® F200 microplate reader (Tecan, Männedorf, Switzerland). Data are presented as a percentage of the values obtained from cells cultured under the same conditions in the absence of clioquinol.

2.3. Caspase-3 activity assay

Caspase-3 activities were measured using EnzChek® Caspase-3 assay kit #2 (Molecular Probes, Eugene, OR). Cell lysates were prepared from 1×10^6 SH-SY5Y cells pretreated in the presence or absence of 50 μmol/L of clioquinol for 8 h. Caspase-3 activities of the lysates were determined by fluorimetric detection at 485/535 nm (excitation/emission) of conversion of the rhodamine 110 bis-(*N*-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide) (Z-DEVD-R110) to fluorescent R110 caused by enzymatic cleavage of DEVD sequence of the substrate. In order to confirm that the observed fluorescence signal was due to caspase-3 activity, the reversible aldehyde inhibitor Ac-DEVD-CHO was used.

2.4. ROS production assay

ROS production by SH-SY5Y cells treated with clioquinol was assessed in two ways: CellMeter™ Fluorimetric Intracellular Total ROS Activity Assay (AAT Bioquest®, Sunnyvale, CA) and 2′,7′-dichlorfluorescein-diacetate (DCFH-DA) assay (Molecular Probes, Eugene, OR). In the former assay, 20,000 SH-SY5Y cells plated in each well of a 96-well culture plate with 100 μl of medium were cultured with indicated concentrations of clioquinol or 100 μmol/L H_2O_2 for 8 h. 20 μl of Amplite™ ROS Green was added for the last one hour. Fluorescent intensity was measured for each well at 535/595 nm (excitation/emission). In the latter, SH-SY5Y cells pretreated with 50 μmol/L of clioquinol for 16 h in chamber slides were stained with MitoTracker® Red CMXRos (Molecular Probes, Eugene, OR), and Hoechst 33342 (Invitrogen, Carlsbad, CA). Immediately after loaded with 20 μmol/L of DCFH-DA, fluorescence was analyzed, using a confocal microscope, C2si (Nikon, Tokyo, Japan) and the image analysis system, NIS Elements (Nikon). Relative ROS signals were calculated by dividing DCF (green) signal intensity by CMXRos (Red) intensity in individual cells. Total 200 cells randomly selected were quantitatively analyzed.

2.5. SOD activity analysis

SOD activity was assayed using the xanthine oxidase/xanthine/cytochrome C method as previously described [14]. Briefly, the control reaction was started by adding 0.005 unit of xanthine oxidase to a reaction mixture containing 10.4 μmol/L cytochrome C, 50.4 μmol/L xanthine, and 99.9 μmol/L EDTA (pH 7.8) at 25 °C in a 3.5 ml cuvette. The absorbance of the cuvette was read every 30 s for up to 5 min at 550 nm, and ΔOD_{550}/min was calculated as rate of the control reaction. ΔOD_{550}/min was obtained for each reaction containing one unit of SOD1 purified from bovine erythrocyte either alone or with indicated concentrations of clioquinol, and percent inhibition of the control reaction rate, which correlated with SOD activity, was determined. Substitution of 100 μg of cell lysates prepared from SH-SY5Y cells for the purified SOD1 allowed the analysis of inhibition of SOD activity in cell lysates by clioquinol. Finally, SOD activity in 100 μg of cell lysates from SH-SY5Y cells cultivated for 20 h in the presence and absence of 50 μmol/L of clioquinol or 100 μmol/L of DDC was analyzed in the same way. Cytosolic protein concentrations were determined with Coomassie Plus (Bradford) assay (Thermo Scientific, Waltham, MA).

2.6. Statistics

Differences among groups of data were assessed using Wilcoxon signed ranks test or one-way ANOVA followed by Bonferroni/Dunn test with $P < 0.05$ as the level of statistical significance.

3. Results

3.1. Clioquinol enhances production of ROS and induces apoptosis in SH-SY5Y cells

Incubation of human neuroblastoma-derived cell line, SH-SY5Y cells, with clioquinol for 24 h reduced their cell viability (Fig. 1A, $P < 0.01$). Reduction of the cell viability was apparent at 10 μmol/L of clioquinol and increased up to 55% at 50 μmol/L in a dose-dependent manner. To determine whether the cell death was accompanied by apoptosis, caspase-3 activities in the cell lysates from SH-SY5Y cells treated with 50 μmol/L of clioquinol for 8 h were measured (Fig. 1B). Caspase-3 activity in SH-SY5Y cells was significantly up-regulated by clioquinol ($P < 0.01$), as shown by generation of higher fluorescent signals from enzymatic cleavage of DEVD sequence in the Z-DEVD-R110 substrate in clioquinol-treated cells and complete abrogation of the signals by addition of the Ac-DEVD-CHO inhibitor ($P < 0.01$).

Next, we decided to examine the relationship between apoptotic cell death and ROS production. After 8-h incubation with 50 μmol/L of clioquinol, intracellular ROS production was significantly enhanced, compared with that of DMSO-treated, control cells (Fig. 1C, $P < 0.01$). Although dose-dependency was not clear, the enhancement of ROS production was apprent at 10 μmol/L of clioquinol, which was the lowest cytotoxic concentration of clioquinol to SH-SY5Y cells. We examined the intracellular localization of ROS using a ROS probe, DCFH-DA. DCF signals were greatly enhanced throughout the cell including the nucleus, mitochondria, and cytosol, when cells were treated with clioquinol (50 μM, 16 h) (Fig. 1D, upper panels). Relative DCF signal to mitochondrial signal was significantly increased in cells with clioquinol treatment (Fig. 1D, lower panel, $P < 0.01$).

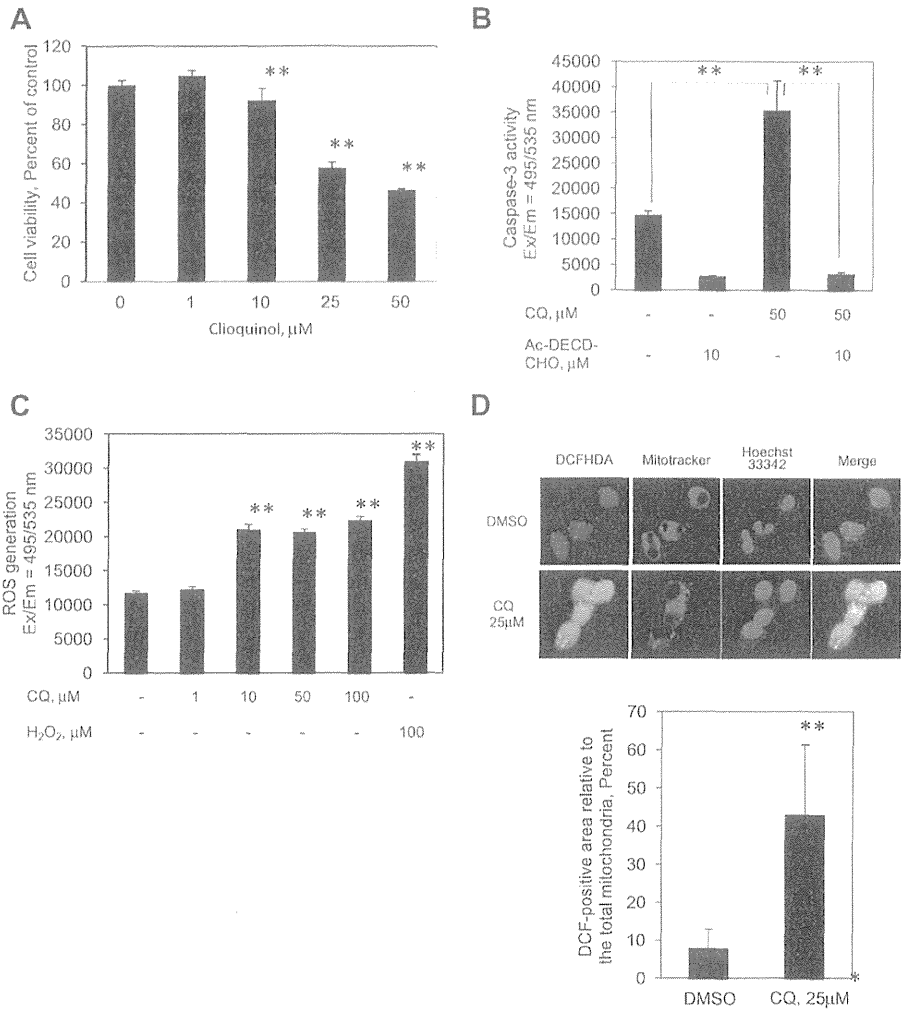

Fig. 1. Clioquinol enhances production of ROS and induces apoptosis in SH-SY5Y cells. (A) cytotoxicity of clioquinol in SH-SY5Y cells. SH-SY5Y cells were plated in a 96-well culture plate at 1×10^4 cells per well and incubated with indicated concentrations of clioquinol for 24 h. Cells were loaded with 10 μl of CellQuanti-Blue™ reagent for the last four hours, and cell viability was analyzed fluorometrically using a microplate reader (**$P < 0.01$, compared with control; one-way ANOVA followed by Bonferroni/Dunn test). (B) Activation of caspase-3 in SH-SY5Y cells treated with clioquinol. Cell lysates were prepared from 1×10^6 SH-SY5Y cells treated with 50 μmol/L of clioquinol for 8 h. Caspase-3 activity in the lysates was determined in a fluorometric assay dependent on DEVD-specific cleavage of the Z-DEVD-R110 substrate. The Ac-DECD-CHO inhibitor was used to confirm that the generated fluorescence signal was due to the activity of caspase-3 (**$P < 0.01$, compared with control; one-way ANOVA followed by Bonferroni/Dunn test). (C) And (D) enhancement of ROS production by clioquinol in SH-SY5Y cells. 2×10^4 SH-SY5Y cells were incubated with indicated concentrations of clioquinol or 100 μM H_2O_2 for 8 h. Intracellular ROS production was determined in a fluorometric analysis, using CellMeter™ Fluorimetric Intracellular Total ROS Activity Assay Kit (C) (**$P < 0.01$, compared with control; one-way ANOVA followed by Bonferroni/Dunn test). SH-SY5Y cells pretreated with 50 μmol/L of clioquinol for 16 h in chamber slides were stained with MitoTracker® Red CMXRos, Hoechst 33342 and 4′,6-diamidino-2-phenylindole (DAPI). Fluorescence microscope images were taken, immediately after the cells were loaded with 20 μmol/L of DCFH-DA (D). Relative ROS signals were calculated by dividing DCF (green) signal intensity by CMXRos (Red) intensity in individual cells for randomly selected two hundred cells. Representative data (mean ± SD) in three to six independent experiments are shown (**$P < 0.01$, compared with control; Wilcoxon signed ranks test). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Clioquinol inhibits SOD1 activity

We analyzed the effect of clioquinol on SOD activity, using the xanthine oxidase/xanthine/cytochrome C method [14]. As shown in Fig. 2A, one unit of SOD1 purified from bovine erythrocytes inhibited by 40.8% the rate of reduction of oxidized cytochrome c by superoxide radicals. Clioquinol suppressed this inhibition to 28.2% at 10 μmol/L ($P < 0.01$), and the suppression by clioquinol was augmented in a dose-dependent manner. Similarly, dose-dependent suppression by clioquinol of SOD activity in cell lysates prepared from SH-SY5Y cells was apparent at 50 μmol/L (Fig. 2B, $P < 0.05$). In order to make sure that inhibition of SOD takes place in cells under treatment with clioquinol, we compared SOD activity in cell lysates from cells cultivated for 20 h in the presence and absence of 50 μmol/L of clioquinol (Fig. 2C). SOD activity in cell lysate from clioquinol-treated cells was significantly suppressed, compared with that of control, DMSO-treated cells (% inhibition of DMSO-treated and clioquinol-treated cells; 46.8% and 30.6%, $P < 0.01$), and the suppression was half as much of that of DDC, a potent copper chelator commonly used as an SOD1 inhibitor.

3.3. Exogenous SOD1 attenuates clioquinol-induced apoptosis

We then determined whether the reduced SOD activity is causal or consequent phenomenon in clioquinol-induced neurotoxicity. When exogenous SOD1 was added into culture medium, ROS

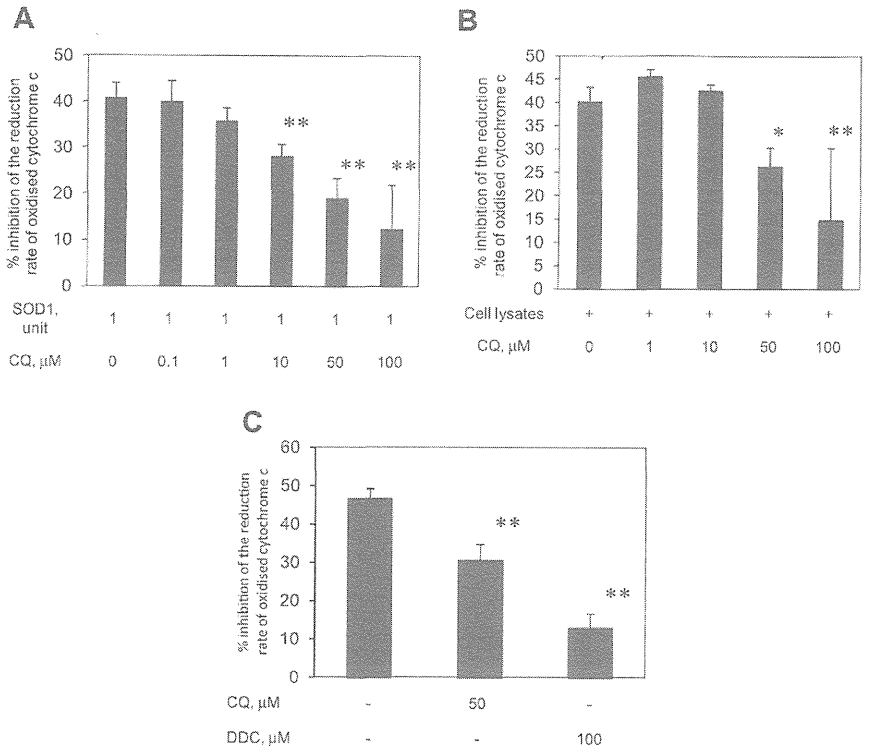

Fig. 2. Effects of clioquinol on SOD1 activity. SOD activity was measured with the xanthine oxidase/xanthine/cytochrome C method and shown as percent inhibition of the control reduction rate of oxidized cytochrome c by the superoxide radicals, as described in Section 2. Clioquinol inhibited enzymatic activity of SOD1 purified from bovine erythrocytes (A) and SOD activity in cell lysates from SH-SY5Y cell (B) in a dose-dependent manner. SOD activity was also inhibited in the cytosol of SH-SY5Y cells cultivated for twenty hours with clioquinol (50 μmol/L) or DDC (100 μmol/L) (C). Representative data (mean ± SD) in three to five independent experiments are shown (*$P < 0.05$, **$P < 0.01$, compared with control SOD activity, using one-way ANOVA followed by Bonferroni/Dunn test).

production induced by clioquinol was significantly reduced (Fig. 3A, $P < 0.01$). Furthermore, the SOD1-treatment significantly improved cell viability (Fig. 3B, $P < 0.01$).

4. Discussion

This study aimed to elucidate the molecular mechanism of clioquinol-induced neurotoxicity, using a human neuronal cell line. Major findings we obtained were as follows: (1) Clioquinol induced apoptotic cell death and augmentation of intracellular ROS production. (2) Clioquinol inhibited enzymatic activity of SOD1. (3) Exogenous SOD1 reduced ROS production and improved cell viability in cells treated with clioquinol. In the present study, clioquinol showed cytotoxicity against the cell at concentrations between 10 and 50 μmol/L. Although plasma concentrations of clioquinol in patients with SMON were not known, it had been

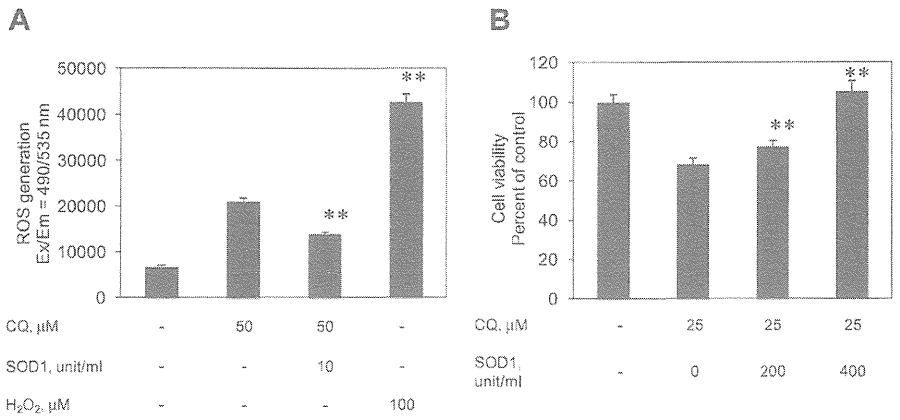

Fig. 3. Exogenous SOD1 inhibits ROS production and cell death induced by clioquinol in SH-SY5Y cells. (A) Simultaneous addition of 10 unit of bovine erythrocyte SOD1 suppressed ROS production in SH-SY5Y cells induced by 50 μmol/L of clioquinol during 8 h of incubation. (B) Exogenously added bovine erythrocyte SOD1 rescued SH-SY5Y cells from clioquinol-induced cell death in a dose-dependent manner (**$P < 0.01$, compared with control treated with clioquinol alone, using one-way ANOVA followed by Bonferroni/Dunn test).

shown in a pharmacokinetic study in healthy subjects that plasma concentrations of clioquinol reached around 30 μg/ml (98 μmol/L) on oral administration of clioquinol 3 × 0.5 g/day for three days [15]. Based on this finding, the cytotoxic concentrations of clioquinol identified and used in this study were considered physiologically relevant.

A previous study, which demonstrated production of malondialdehyde and cell death in neurons in clioquinol-treated murine cortical cultures, suggested a pro-oxidant effect of clioquinol and its association with neurotoxicity [9]. Since then, several fragments of evidence for clioquinol-induced neurotoxicity have been reported, including induction of DNA double-strand breaks (DSBs) followed by activation of p53 signaling, inhibition of Trk autophosphorylation and RNA synthesis induced by nerve growth factor, formation of clioquinol zinc chelates acting as a mitochondrial toxin, and disturbance of vitamin B12 homeostasis in the brain [7,8,16–18]. Some of these mechanisms are closely associated with oxidative stress. However, there are few reports showing the direct relationship with ROS. Our study is the first to show that a pro-oxidant effect of clioquinol is mediated by enhanced ROS production via SOD1 inhibition. The finding is supported by a report demonstrating induction of neuronal apoptosis by chronic inhibition of SOD with either antisense oligodeoxynucleotides or DDC in murine spinal cord organotypic cultures [19].

It is widely believed that ROS are involved in induction of apoptosis as key players under physiologic and pathologic conditions. Excess intracellular ROS are sufficient to trigger apoptosis by initiating the redox-sensitive ASK1/JNK pathway or opening mitochondrial permeability transition pore (PTP) through oxidative modification of PTP component proteins [20,21]. Some studies have reported the existence of ROS-independent apoptosis, which was induced by chemical agents, such as gossypol, myricetin, and 7,8-dihydroxy-4-methylcoumarin [22–24]. In apoptotic cells, ROS released from mitochondria, following the original stimulus, in turn, facilitate the apoptotic process [25,26]. Therefore, ROS may cause apoptosis and result from apoptosis. There was a possibility that enhanced ROS production we found in clioquinol-treated cells was not a cause of apoptosis, just an epiphenomenon of induction of apoptosis. We ruled out this possibility by showing that both cell death and enhancement of ROS production in clioquinol-treated cells were prevented by exogenous purified SOD1.

SOD, that requires metal cofactor for their enzymatic activities, catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide [27]. In human, it is classified into three classes of SOD (SOD1–3), depending on the cofactor. SOD1 and SOD3, which are located in the cytoplasm and the extracellular matrix respectively, require copper and zinc, whereas SOD2 in the mitochondria requires manganese. It plays a crucial role for cellular antioxidant defense, together with catalase and glutathione peroxidase (GPX) [28]. Oxidative stress is generated from an imbalance between ROS production and scavenging caused by impairment of the antioxidant system and is involved in almost all disease, degenerative, vascular, and inflammatory. Recent studies demonstrated that impairment of SOD activities are related to neurological disorders [29,30]. Loss of function of DJ-1 decreases SOD1 expression, making dopaminergic cell more susceptible to ROS-induced cell death, in Parkinson's disease [29]. Furthermore, excess superoxide generated from SOD1 deficiency causes spontaneous intracranial hemorrhage through activation of matrix metaloproteinase-9 [30]. These findings as well as the present results suggest that oxidative stress mediated by SOD1 inhibition underlies the pathogenesis of clioquinol-induced neuronal damage, to which attention should be paid, when clioquinol or its derivatives are used for treatment of neurodegenerative disorders, such as AD.

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3 神経難病患者の特性 ―分類と特徴―

1 難病とは

難病

難病とは，英訳するとintractable disease（直訳は治りにくい疾病）で，日本独特の施策事業上の名前であり，海外にはこのような考え方は知り得た範囲ではない．「難病の歴史と今後」の項で詳述するが，1972（昭和47）年の厚生省の難病対策要綱のなかで，難病とは治療方法に乏しく，後遺症が多く出現し，療養生活上患者本人や家族への負担の多大なる疾病群と定義されている．

2 神経難病とは

神経難病

難病のなかで神経系，および筋肉系が障害される疾患群である．いずれの疾患においても運動器が障害されることが多く，歩行困難による自力での移動障害，嚥下困難や上肢の運動麻痺による摂食障害など，介護者にとってより負担が多くなる．また，患者自身も援助してもらっていることに精神的な負担が多いことをしばしば訴える．しかし，障害者福祉の世界でよく言われる「ノーマライゼーション」，すなわち障害の有無にかかわらず人間が平等に権利と義務を分担する思想が主流となる時代を，障害を持ちながら主体的に生き抜くことのモデルケースにすべき疾患が多いともいえる．

3 難病の歴史と今後

A スモン（SMON：subacute myelo-optico-neuropathy）が契機

スモン

昭和30年代に入り，スモンが社会的問題となった．スモンは腹部症状とともに亜急性に視神経炎と脊髄炎が加わり，重症例は全盲，完全両下肢麻痺などを呈する．昭和40年代に入り，国会でスモンなど患者数が少なく研究対象となりにくい疾病への対策が提起される．1971（昭

和46）年に国会議員による「難病対策議員懇談会」が発足．1972（昭和47）年にスモンは整腸剤の「キノホルム」中毒と断定．同年10月，時の厚生省はスモンの原因究明方法を参考とし，「難病対策要綱」を作成し，難病対策を開始した．

B 支援の内容

原因究明（特定疾患治療研究事業，難治性疾患克服研究事業など），絶え間ない支援の仕組みの構築（難病特別対策推進事業，重症難病患者入院施設確保事業など）がおもな事業内容である．治療研究事業は患者の医療費の自己負担解消事業であり，医師が患者に「特定疾患の手続きを申請してください」という事業は，この事業を指す．

C 特定疾患治療研究事業

1972年発足当時の対象疾患はスモン，ベーチェット病，多発性硬化症，重症筋無力症，全身性エリテマトーデスの５疾患で，患者数も少なかった．2011（平成23）年度時点では図1に示すように，56疾患で，患者数も78万人と膨大なものとなった．事業主体は都道府県で，すなわち費用を国と都道府県で折半することになる．都道府県の経費不足が深刻な段階となり，また希少疾患が対象であると要綱にはうたわれているが，実際は対象から外れている希少疾患も多く，不公平さなど多くの問題点が噴出し，新たな難病支援方法の構築が必要になってきた．

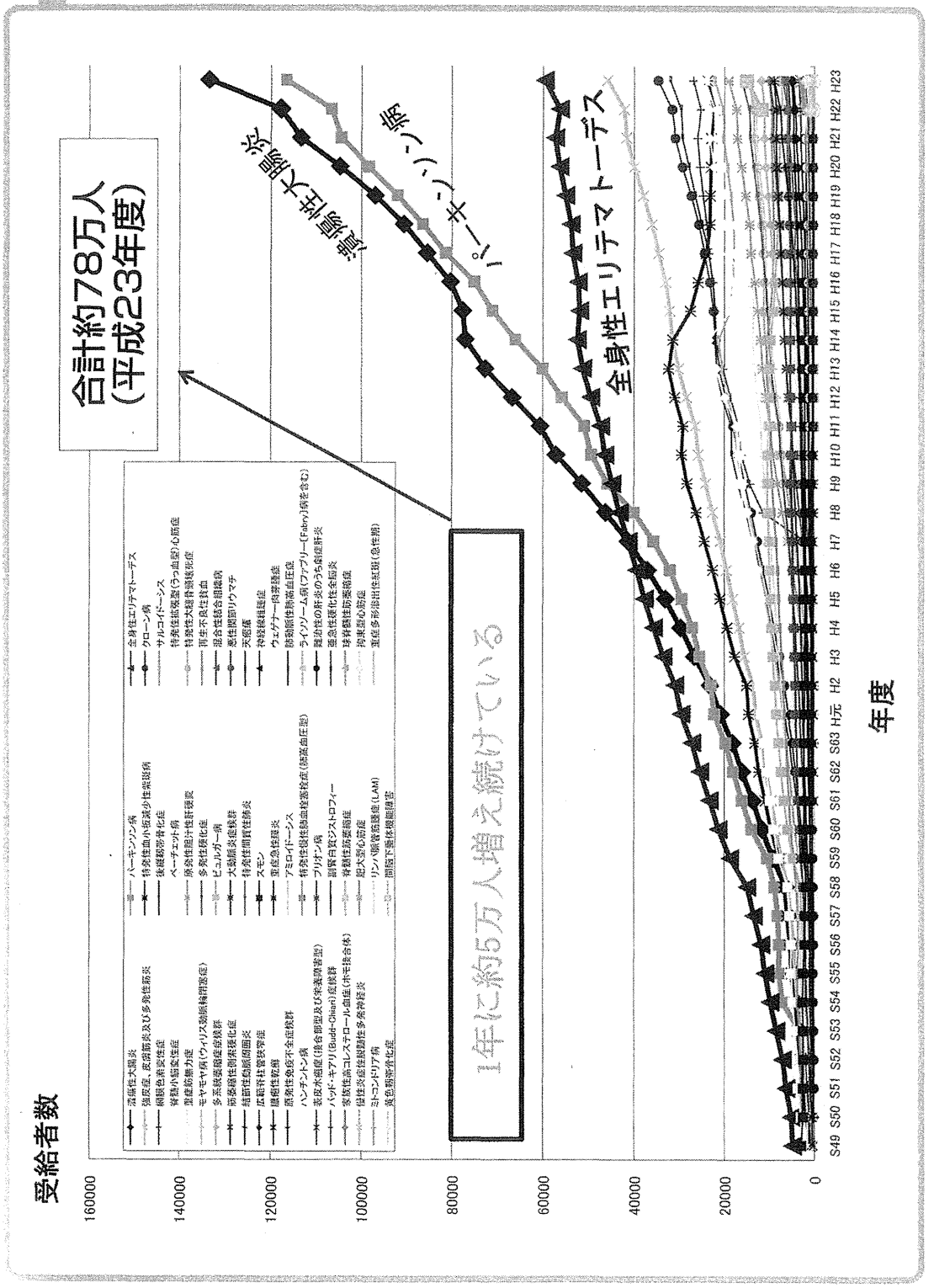
D 難病新法の成立

難病新法

2014年５月に難病新法が成立した．難病患者の社会参加の機会確保および地域社会における共生が基本理念として掲げられるとともに，公費負担による医療費助成などの措置が法律に基づく制度として講じられる．

難病政策における何十年に１回の大変革であるが，同年８月４日に厚生労働省より現時点での指定難病110疾病が提示された（表1）．今までは重症難病患者の在宅療養支援が中心であったが，難病を持ちながら自立している患者や，就労支援など多彩なサービスが求められる．また，疾病数の増加や支援経験の乏しい希少難病も多く含まれていることなど，医療従事者としては新たな支援システムづくりが要求される．しかし，難病の基本的な受け止め方を変える必要はなく，病院診療と在宅診療を含めトータルに支援する必要がある疾病と考えて対処すれば大きな間違いを犯すことはない．

図 1 特定疾患患者数（全国）



特定疾患治療研究事業の対象疾患と受給者数の年代ごとの推移.

（文献 1 より引用）

表 1 指定難病110疾病

難病の患者に対する医療等に関する法律第5条第1項に規定する指定難病

疾病番号	病名	備考(※)	疾病番号	病名	備考(※)
1	球脊髄性筋萎縮症	特定疾患	56	ベーチェット病	特定疾患
2	筋萎縮性側索硬化症	特定疾患	57	特発性拡張型心筋症	特定疾患
3	脊髄性筋萎縮症	特定疾患	58	肥大型心筋症	特定疾患
4	原発性側索硬化症		59	拘束型心筋症	特定疾患
5	進行性核上性麻痺	特定疾患	60	再生不良性貧血	特定疾患
6	パーキンソン病	特定疾患	61	自己免疫性溶血性貧血	
7	大脳皮質基底核変性症	特定疾患	62	発作性夜間ヘモグロビン尿症	
8	ハンチントン病	特定疾患	63	特発性血小板減少性紫斑病	特定疾患
9	神経有棘赤血球症		64	血栓性血小板減少性紫斑病	
10	シャルコー・マリー・トゥース病		65	原発性免疫不全症候群	特定疾患
11	重症筋無力症	特定疾患	66	IgA 腎症	
12	先天性筋無力症候群		67	多発性嚢胞腎	
13	多発性硬化症／視神経脊髄炎	特定疾患	68	黄色靱帯骨化症	特定疾患
14	慢性炎症性脱髄性多発神経炎／多巣性運動ニューロパチー	特定疾患	69	後縦靱帯骨化症	特定疾患
			70	広範脊柱管狭窄症	特定疾患
15	封入体筋炎		71	特発性大腿骨頭壊死症	特定疾患
16	クロウ・深瀬症候群		72	下垂体性ADH分泌異常症	特定疾患
17	多系統萎縮症	特定疾患	73	下垂体性TSH分泌亢進症	特定疾患
18	脊髄小脳変性症（多系統萎縮症を除く）	特定疾患	74	下垂体性PRL分泌亢進症	特定疾患
			75	クッシング病	特定疾患
19	ライソゾーム病	特定疾患	76	下垂体性ゴナドトロピン分泌亢進症	特定疾患
20	副腎白質ジストロフィー	特定疾患			
21	ミトコンドリア病	特定疾患	77	下垂体性成長ホルモン分泌亢進症	特定疾患
22	もやもや病	特定疾患	78	下垂体前葉機能低下症	特定疾患
23	プリオン病	特定疾患	79	家族性高コレステロール血症（ホモ接合体）	特定疾患
24	亜急性硬化性全脳炎	特定疾患			
25	進行性多巣性白質脳症		80	甲状腺ホルモン不応症	
26	HTLV-1関連脊髄症		81	先天性副腎皮質酵素欠損症	
27	特発性基底核石灰化症		82	先天性副腎低形成症	
28	全身性アミロイドーシス	特定疾患	83	アジソン病	
29	ウルリッヒ病		84	サルコイドーシス	特定疾患
30	遠位型ミオパチー		85	特発性間質性肺炎	特定疾患
31	ベスレムミオパチー		86	肺動脈性肺高血圧症	特定疾患
32	自己貪食空胞性ミオパチー		87	肺静脈閉塞症／肺毛細血管腫症	特定疾患
33	シュワルツ・ヤンペル症候群		88	慢性血栓塞栓性肺高血圧症	特定疾患
34	神経線維腫症	特定疾患	89	リンパ脈管筋腫症	特定疾患
35	天疱瘡	特定疾患	90	網膜色素変性症	特定疾患
36	表皮水疱症	特定疾患	91	バッド・キアリ症候群	特定疾患
37	膿疱性乾癬（汎発型）	特定疾患	92	特発性門脈圧亢進症	
38	スティーヴンス・ジョンソン症候群	特定疾患	93	原発性胆汁性肝硬変	特定疾患
39	中毒性表皮壊死症	特定疾患	94	原発性硬化性胆管炎	
40	高安動脈炎	特定疾患	95	自己免疫性肝炎	
41	巨細胞性動脈炎		96	クローン病	特定疾患
42	結節性多発動脈炎	特定疾患	97	潰瘍性大腸炎	特定疾患
43	顕微鏡的多発血管炎	特定疾患	98	好酸球性消化管疾患	
44	多発血管炎性肉芽腫症	特定疾患	99	慢性特発性偽性腸閉塞症	
45	好酸球性多発血管炎性肉芽腫症		100	巨大膀胱短小結腸腸管蠕動不全症	
46	悪性関節リウマチ	特定疾患	101	腸管神経節細胞僅少症	
47	バージャー病	特定疾患	102	ルビンシュタイン・テイビ症候群	
48	原発性抗リン脂質抗体症候群		103	CFC症候群	
49	全身性エリテマトーデス	特定疾患	104	コステロ症候群	
50	皮膚筋炎／多発性筋炎	特定疾患	105	チャージ症候群	
51	全身性強皮症	特定疾患	106	クリオピリン関連周期熱症候群	
52	混合性結合組織病	特定疾患	107	全身型若年性特発性関節炎	
53	シェーグレン症候群		108	TNF受容体関連周期性症候群	
54	成人スチル病		109	非典型溶血性尿毒症症候群	
55	再発性多発軟骨炎		110	ブラウ症候群	

（※）備考欄の特定疾患は、現行の医療費助成制度（特定疾患治療研究事業）においても対象となっている疾病。現行制度の疾病名と異なるものもある（疾病名対比表を参照）。

（文献2より引用）

4 神経難病の分類

難病の一覧（表1）の中で，1番の球脊髄性筋萎縮症から34番の神経線維腫症までが神経内科で扱う疾病である．

神経内科疾患は，脳血管障害や脳炎などの急性期疾患から，神経変性疾患などの慢性期疾患，てんかんや頭痛などの機能性疾患まで，扱う疾患は幅広い．このうち本稿の主題である神経難病は，定義どおり，原因が明らかではない筋萎縮性側索硬化症，パーキンソン病，脊髄小脳変性症などの変性疾患が多い．しかし，多発性硬化症や重症筋無力症などの難治性の自己免疫疾患，さらにハンチントン病や副腎白質ジストロフィーなどの遺伝子の異常で起こる疾患も含まれている．

5 神経難病の特徴

A 症状は進行性である

症状が徐々に，いつとはなしに出現することは，脳梗塞など急性に症状が出現するものと対照をなす．そして，いったん症状が出現し始めると，疾患による違いや，同一疾患でも個々の症例によって相違はあるが，進行することが多い．

B 補充療法とケアが中心

運動器が障害されることが多く，下肢の筋力低下が進行し，歩行不能になった場合は車いす生活となる．呼吸筋が障害されると，人工呼吸器装着が必要となる．嚥下筋が障害されると，経鼻胃管の挿入による，あるいは胃瘻による栄養補給が必要になる．治療法が未確立なため，医療は上記のような補充療法とケアにとどまっている．しかし，物は考えようで，看護職にとってはやりがいのある分野かもしれない．

補充療法

C 在宅療法では，家族による介護が中心

家族による介護が中心になるが，本人，家族の精神的，肉体的負担は大きい．また，遺伝子の異常による疾患では，介護者自身がat risk（保因者：遺伝子の異常をもっており，将来発症する可能性がある者）のこともある．介護者は自分自身の発症の不安と闘いながら，家族を支援す

ることになる．介護者への支援も重要なことである．

さらに，遺伝性疾患では，個人情報保護など，社会的，文化的，倫理的問題を考慮する必要もある．

D 神経難病のケアは，緩和ケア

がんの終末医療における緩和ケアと比較し，死に方を考えるのではなく，より充実した人生をつくって生き抜くことを考えておく必要がある．極端な例では，たとえば筋萎縮性側索硬化症は死亡する疾患ではないため，人工呼吸器装着を希望せずに死亡するということは，患者自身が，自分の生命観で死を選んだともいえる．もちろん，その場合でも医療従事者は，患者自身とともに語り合い，お互いの人生を高めるように努力する．

E 医療依存度の高い疾患が多い

筋萎縮性側索硬化症で人工呼吸器装着を選択した場合，施設医療と在宅医療の両者で切れ目のない支援が必要になる．気管切開部分からの痰の吸引について，施設医療では看護師が専門的に実施するが，在宅医療に移行した場合は，この医療依存度の高い処置を家族が行う場合が多い．入院中の家族への指導は，看護師の重要な務めである．

しかし，患者自身がこのような家族への負担を過度に考え，意志の自律（ほかからの支配や制限を受けず，自分自身で立てた規範で行動すること）に反し，人工呼吸器装着を拒否することが多いのも事実である．

F 補充療法選択におけるICの難しさ

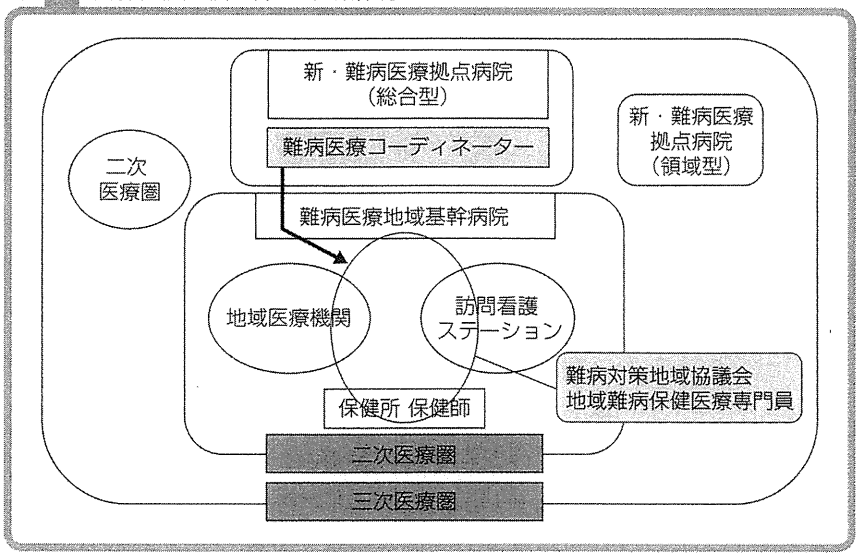
人工呼吸器装着の有無についてのIC（インフォームド・コンセント）などが最たるものである．共感（empathy），すなわち同情ではなく，医師と患者が互いに聞く耳をもち，語り合うことにより，互いの人生を認め合い充実させようとする心掛けが大切である．

G ネットワークによる支援が重要

神経難病はケアと在宅療養が中心となるが，施設医療と在宅医療の切れ目のない連携構築でのバックアップが重要となる．1998（平成10）年に厚生労働省の医療施設整備事業に伴い，各都道府県に難病の拠点病院が指定された．拠点病院に事務局を置き，神経難病医療ネットワーク事業が展開されてきた．

神経難病医療ネットワーク事業

図2 難病新法成立後の新たな医療体制



三次医療圏に1カ所設置された新・難病医療拠点病院を中心に，難病医療コーディネーター，地域難病保健医療専門員がネットワーカーとなり連携を図る．

ネットワークで重要なことは，ボランティア精神で働くネットワーカーであるが，難病医療専門員（看護師）がこれに相当する．地域の保健所・保健センターと協力して，ケアシステムの構築を中心に難病患者の療養環境の向上に成果を上げてきた．

H 難病新法で難病支援の持続ある発展を期待

先述したように，2014年5月の難病新法の成立で，図2に示すような新たな難病支援の連携が構築される予定である．難病医療は病院診療と在宅診療のトータルな支援を必要とする．支援の仕組みとしてたいへん素晴らしいものであり，内容の充実を期待する．

主役は患者と看護師 check!

神経難病医療はケアが中心．患者を介護しながら語り合うことで，お互いの人生を充実させよう．

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(狭間敬憲)

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