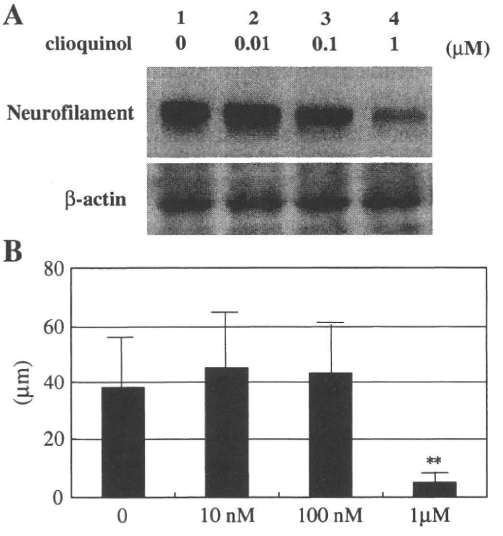

Fig. 2 – (A) Neurofilament expression in NGF-stimulated cells by western blotting. The cells were stimulated with 50 ng/ml of NGF and were cultured with various concentrations of clioquinol for 48 h. Cell lysates were applied for western blotting with anti-neurofilament antibody or anti-β-actin antibody. Note the decrease of neurofilament expression in higher concentration of clioquinol in contrast to the stable expression of β-actin. (B) NGF-stimulated cells were cultured with different amount of clioquinol for 24 or 48 h. Neurite retraction by clioquinol was quantitated by measurement of neurite length manually in phase contrast micrographs. Each 100 cells from three independent wells were evaluated. Bars and error bars represent mean ± SEM. Statistical analysis by Student's t-test was performed, $^{**}p<0.01$.

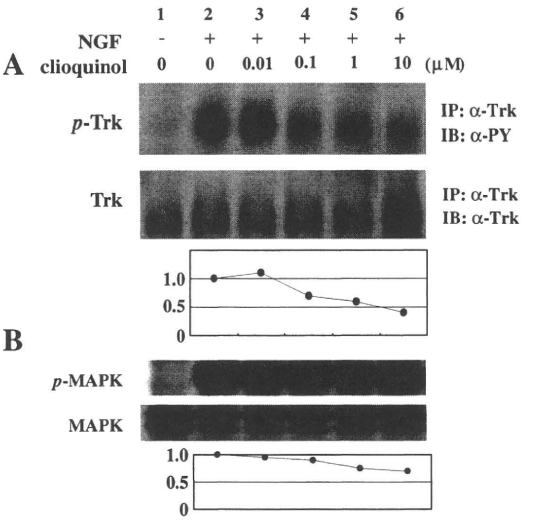

Fig. 4 – Concentration-dependent effects of clioquinol on Trk and MAPK phosphorylation. Cells were incubated with clioquinol for 1 h at various concentrations, then the cells were stimulated with 50 ng/ml of NGF for 5 min. Cell-free lysates were immunoprecipitated with α-Trk. Trk immunoprecipitates were subjected to SDS–PAGE and were immunoblotted with α-PY or α-Trk (A). Cell-free lysates were also subjected to SDS–PAGE and immunoblotted with anti-phosphospecific MAPK antibody (B) and anti-MAPK antibody (B), respectively. The cells in lane 1 were not stimulated with NGF and the cells from lane 2 to 6 were stimulated with NGF (50 ng/ml) for 5 min. Small graphs showed relative ratio of Trk autophosphorylation or MAPK phosphorylation. Phosphorylated Trk or MAPK at each concentration of clioquinol was adjusted with total Trk protein or MAPK protein, respectively, and was compared as a ratio.

To further confirm the inhibition of NGF-induced Trk autophosphorylation by clioquinol, MAPK (mitogen-activated protein kinase) phosphorylation response to NGF was also examined. MAPK is a serine/threonine kinase, which is located in the downstream of Trk signal transduction pathway and a key molecule to convey a signal to the nucleus. As shown in Fig. 3, NGF treatment caused MAPK phosphorylation (lane 2).

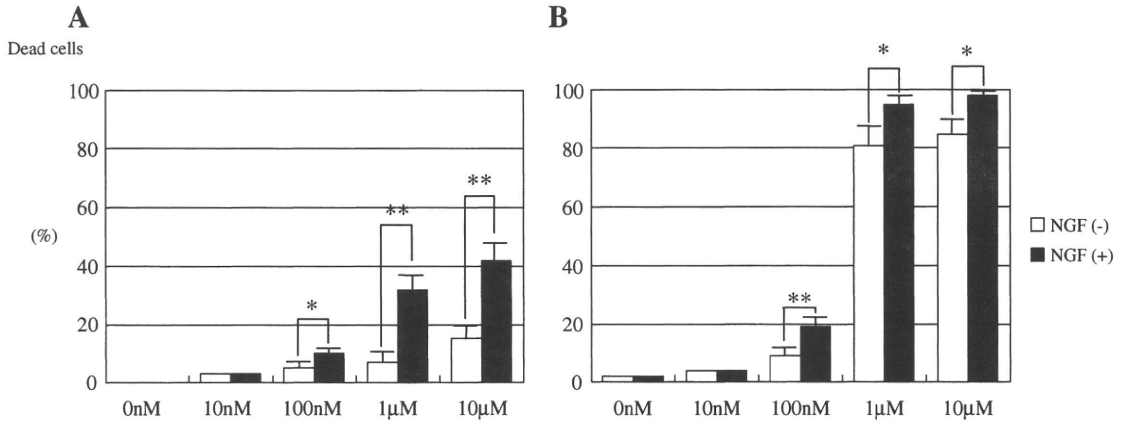

Fig. 3 – Cell viability assay by trypan blue staining. Cells cultured with or without NGF were incubated with various concentrations of clioquinol for 24 or 48 h. Dead cells were counted by trypan blue staining. Each experiment was performed in triplicate. Bars and error bars represent mean ± SEM. Note the NGF-stimulated cells are significantly vulnerable to clioquinol. Statistical analysis by Student's t-test was performed, $^{*}p<0.05$; $^{**}p<0.01$.

The addition of clioquinol in culture medium caused a reduction of MAPK phosphorylation response similar to the Trk autophosphorylation response (lanes 3–6).

3. Discussion

Extensive epidemiological studies demonstrated that intake of clioquinol causes SMON and the prohibition of clioquinol as a medicine in 1970 dramatically decreased the number of new cases of SMON in Japan (Konagaya et al., 2004). In contrast to the epidemiological success, pathogenic mechanism of clioquinol neurotoxicity is still unknown even after 30 years of discontinuation of clioquinol. In this study, to elucidate the pathogenic mechanism of clioquinol, we focused on the NGF-Trk-mediated signal transduction pathway and demonstrated that clioquinol inhibits autophosphorylation of Trk receptor on a neuronal cell line. Clioquinol inhibited Trk autophosphorylation in a concentration-dependent manner and it was further confirmed that the phosphorylation of MAPK, which is a key molecule located in the downstream of NGF-Trk signal transduction pathway, was also inhibited by clioquinol. Under microscope, clioquinol caused neurite retraction in NGF-stimulated cells. In addition, clioquinol caused cell death both in NGF-stimulated and non-stimulated cells. However, NGF-stimulated cells (differentiated cells) were more vulnerable to clioquinol than non-stimulated cells (undifferentiated naïve cells).

In this study, 1 μM was a critical concentration of clioquinol toxicity to PCtrk cells. It is compatible with the previous observation in the animal experiments of SMON, i.e., plasma levels of clioquinol were approximately 0.5 to 5 μg/ml (1.7 to 17 μM) (Matsuki et al., 1997). Recently clioquinol has been the focus of attention as a potential drug for malignancy, Alzheimer's disease, and Huntington's disease (Chen et al., 2007; Cherny et al., 2001; Nguyen et al., 2005; Ritchie et al., 2003). For these new indications, further understanding of clioquinol neurotoxicity is necessary to avoid potential side effects of this drug.

Heretofore, the molecular mechanisms of clioquinol-induced neurotoxicity have been proposed. Among various potential mechanisms of clioquinol neurotoxicity postulated, metal ion chelating activity of clioquinol, notably Zn^{2+}, Cu^{2+}, Co^{2+}, and Fe^{3+}, is one of the most attractive mechanism because based upon this chelating activity, clioquinol is started to use as a therapeutic drug for malignancy, Alzheimer's disease, and Huntington's disease, vice versa. Clioquinol, in the presence of zinc, is reported to be converted to a potent mitochondrial toxin (Arbiser et al., 1998). Since clioquinol has been shown to cause increased systemic absorption of zinc in humans, it is likely that clioquinol-zinc chelate was present in appreciable levels in patients with SMON and may be the ultimate causative toxin of SMON (Arbiser et al., 1998). Clioquinol can form powerful lipophilic chelates with divalent cations, and this characteristic has led to the speculation that clioquinol is able to function as a carrier of heavy metals to the CNS which could cause toxicity in nerve tissue (Ohtsuka et al., 1982; Yagi et al., 1985; Yassin et al., 2000). Clioquinol injection in mice resulted in a rapid loss of synaptic zinc. Immediate early gene transcription factor, c-Fos, was induced in the hippocampal region and other parts of telencephalon and subsequently cell death was observed in these areas (Ismail et al., 2008).

What is the molecular mechanism for the strong inhibition of clioquinol on Trk-mediated intracellular survival pathway? Zinc itself has been known to alter the conformation and to inhibit the biological activity of neuropeptides related to neuronal survival, including NGF (Ross et al., 1997). However, in the present experimental conditions, zinc was free in the culture medium. Therefore, zinc-related mechanism for the inhibition of Trk autophosphorylation seems unlikely. Trk has an intrinsic tyrosine kinase activity, which is located in the cytoplasmic domain. The initial step of the intracellular signal transduction of NGF is believed to be activated by the autophosphorylation on tyrosine residues. Although the clear molecular mechanism of clioquinol-induced impairment of the Trk receptor function remains to be elucidated, Trk-initiated intracellular signaling pathway of NGF is an essential pathway for neuronal survival and differentiation in vivo. The evidence that differentiated cells stimulated with NGF were more vulnerable than undifferentiated cells in this study is compatible with the prominent post-mitotic neuronal cell damage in SMON. Therefore, it is quite reasonable to assume that the failure of this important cell survival pathway would result in the fatal impacts on the normal biology of the neurons. Thus, clioquinol-induced impairment of Trk autophosphorylation response in vitro culture system would implicate the new possible molecular mechanism for the development of the pathological states observed in patients with SMON. Moreover, this hypothesis suggests a new therapeutic strategy employing NGF for the treatment of many patients suffering from intractable residual symptoms such as pain and neuronal dysfunctions.

4. Experimental procedures

4.1. Cell culture

PC12 cells transformed with human *trk* complimentary DNA (PCtrk cells) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% horse serum, 5% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin (Mutoh et al., 2000). Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) was purchased from Sigma (St. Louis, MO) and was dissolved in 100% DMSO at a final concentration of 10 mM as a stock solution. The stock solution was further diluted for various experiments. To exclude the cellular toxicity of DMSO to PCtrk cells, the vehicle containing equivalent amount of DMSO was examined. We confirmed that cells were not affected by DMSO up to 0.2% concentration.

4.2. Neurite retraction

We examined the effect of clioquinol on NGF-stimulated neurite retraction of PCtrk cells. PCtrk cells were stimulated with 50 ng/ml of NGF in serum-free medium overnight, then the neurite-extended cells were cultured with 50 ng/ml of NGF and various concentration of clioquinol (from 10 nM to 10 μM) for 48 h. The cells were observed under phase contrast

microscope. To examine the effect on neurite-extended PCtrk cells, neurofilament expressions were determined by western blotting. Western blotting with anti-neurofilament antibody (Biomol International, Plymouth Meeting, PA) or anti-β-actin antibody (Cell Signaling Technology, Danvers, MA) was performed to further confirm the effect of clioquinol on neurite-extended cells. Detailed procedures of western blotting were described in section 4.3.

Furthermore, to quantitate the morphological effects on neurite-extended cells, phase contrast micrographs took from typical areas of these cultures and the length of neurites was measured manually. Each 100 cells from three independent wells were evaluated. Statistical analysis by Student's *t*-test was performed.

4.3. *Trk autophosphorylation and MAPK phosphorylation*

Cells were preincubated with serum-free medium for 1 h at 37 °C, and the cells were incubated in the presence of clioquinol at various concentrations (from 10 nM to 10 μM) for 1 h. Then, the cells were stimulated with 50 ng/ml of NGF for 5 min to examine the effect of clioquinol on NGF-induced Trk autophosphorylation. After stimulation, the cells were collected with chilled phosphate-buffered saline and lysed with lysis buffer (20 nM HEPES, pH7.2/1% Nonidet P-40/10% (vol./vol.) glycerol/50 mM NaF/1 mM phenylmethylsulphonyl fluoride (PMSF)/1 mM Na_3VO_4/10 μg of leupeptin per ml). The cell-free lysates were normalized for protein (1 mg/ml) and immunoprecipitated with anti-Trk antibody (α-Trk; Santa Cruz Biotech Inc, Santa Cruz, CA). The Trk immunoprecipitates were separated by sodium dodecyl sulfate–polyacrylamide electrophoresis (SDS–PAGE) under reducing conditions on 7–14% gradient acrylamide gels, which was followed by blotting on polyvinylidene difluoride (PVDF) membranes. Tyrosine phosphorylation of Trk was detected with an anti-phosphotyrosine antibody (α-PY; Upstate Biotechnology Inc., Waltham, MA) and Trk was detected with α-Trk. The positive bands were detected with horseradish peroxidase-conjugated secondary antibodies using enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) (Asakura et al., 2007). The cell-free lysates were also subjected to SDS–PAGE and immunoblotted with anti-phosphospecific mitogen-activated protein kinase (p-MAPK) antibody (α-p-MAPK; New England Biolabs, Tokyo, Japan) or anti-MAPK antibody (α-MAPK; New England Biolabs). Semi-quantitative analyses of Trk autophosphorylation and MAPK phosphorylation were performed by measuring the density of each band by densitometer (Shimadzu, Kyoto, Japan). Phosphorylated Trk or MAPK at each concentration of clioquinol was adjusted with total Trk protein or MAPK protein and was compared as a ratio (Mutoh et al., 1995).

4.4. *Cell survival assay*

To detect the cell survival the cells were incubated with trypan blue (Sigma). The cells were seeded onto 24-well plates and were cultured with or without NGF (50 ng/ml) in serum-free medium. Then, the cells were cultured with various concentration of clioquinol for another 24 or 48 h. Under phase contrast microscope, the number of viable (unstained) and dead (stained) cells was counted. Each experiment was performed in triplicate and statistical analysis by Student's t-test was done.

Acknowledgments

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REFERENCES

Arbiser, J.L., Kraeft, S.-K., van Leeuwen, R., Hurwitz, S.J., Selig, M., Dickersin, G.R., Flint, A., Byers, H.R., Chen, L.B., 1998. Clioquinol-zinc chelate: a candidate causative agent of subacute myelo-optic neuropathy. Mol. Med. 4, 665–670.

Asakura, K., Murayama, H., Himeda, T., Ohara, Y., 2007. Expression of L* protein of Theiler's murine encephalomyelitis virus in the chronic phase of infection. J. Gen. Virol. 88, 2268–2274.

Chen, D., Cui, Q.C., Yang, H., Barrea, R.A., Sarkar, F.H., Sheng, S., Yan, B., Reddy, P.V., Ping Dou, Q., 2007. Clioquinol, a therapeutic agent for Alzheimer's disease, has proteasome-inhibitory, androgen receptor-suppressing, apoptosis-inducing, and anti-tumor activities in human prostate cancer cells and xenografts. Cancer Res. 67, 1636–1644.

Cherny, R., Atwood, C., Xilinas, M., Gray, D., Jones, W., McLean, C., Barnham, K., Volitakis, I., Fraser, F., Kim, Y., Huang, X., Goldstein, L., Moir, R., Lim, J., Beyreuther, K., Zheng, H., Tanzi, R., Masters, C., Bush, A., 2001. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 30, 665–676.

Ismail, T., Mauerhofer, E., Slomianka, L., 2008. The hippocampal region of rates and mice after a single i.p. dose of clioquinol: loss of synaptic zinc, cell death and c-Fos induction. Neuroscience 157, 697–707.

Kaplan, D.R., Miller, F.D., 2000. Neurotrophin signal transduction in the nervous system. Curr. Opin. Neurobiol. 10, 381–391.

Konagaya, M., Matsumoto, A., Takase, S., Mizutani, T., Sobue, G., Konishi, T., Hayabara, T., Iwashita, H., Ujihira, T., Miyata, K., Matsuoka, Y., 2004. Clinical analysis of longstanding subacute myelo-optico-neuropathy: sequelae of clioquinol at 32 years after its ban. J. Neurol. Sci. 218, 85–90.

Matsuki, Y., Yoshimura, S., Abe, M., 1997. SMON and pharmacokinetics of chinoform with special reference to animal species difference. Yakugaku Zasshi 7, 936–956 [in Japanese with English abstract].

Mutoh, T., Tokuda, A., Miyadai, T., Hamaguchi, M., Fujiki, N., 1995. Ganglioside GM1 binds to the Trk protein and regulates receptor function. Proc. Natl. Acad. Sci. U. S. A. 92, 5087–5091.

Mutoh, T., Hamano, T., Tokuda, A., Kuriyama, M., 2000. Unglycosylated Trk protein does not co-localized nor associate with ganglioside GM1 in stable clone of PC12 cells overexpressing Trk (PCtrk cells). Glycoconj. J. 17, 233–237.

Nakae, K., Yamamoto, S., Shigematsu, I., Kono, R., 1973. Relation between subacute myelo-optic neuropathy (S.M.O.N.) and clioquinol: nationwide survey. Lancet 1, 171–173.

Nguyen, T., Hamby, A., Massa, S.M., 2005. Clioquinol down-regulates mutant huntintin expression in vitro and mitigates pathology in a Huntington's disease mouse model. Proc. Natl. Acad. Sci. U. S. A. 102, 11840–11845.

Ohtsuka, K., Ohishi, N., Eguchi, G., Yagi, K., 1982. Degeneration of

retinal neuroblasts by chinoform-ferric chelate. Experientia 38, 120–122.
Ritchie, C.W., Bush, A.I., Mackinnon, A., Macfarlane, S., Mastwyk, M., MacGregor, L., Kiers, L., Cherny, R., Li, Q.X., Tammer, A., Carrington, D., Mavros, C., Volitakis, I., Xilinas, M., Ames, D., Davis, S., Beyreuther, K., Tanzi, R.E., Masters, C.L., 2003. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. Arch. Neurol. 60, 1685–1691.
Ross, G.M., Shamovsky, I.L., Lawrance, G., Solc, M., Dostaler, S.M., Jimmo, S.L., Weaver, D.F., Riopelle, R.J., 1997. Zinc alters conformation and inhibits biological activities of nerve growth factor and related neurotrophins. Nat. Med. 3, 872–878.
Tateishi, J., 2000. Subacute myelo-optico-neuropathy: clioquinol intoxication in humans and animals. Neuropathology 20, S20–S24.
Thoenen, H., 1995. Neurotrophins and neuronal plasticity. Science 270, 593–598.
Tsubaki, T., Honma, Y., Hoshi, M., 1971. Neurological syndrome associated with clioquinol. Lancet 1, 696–697.
Yagi, K., Ohtsuka, K., Ohishi, N., 1985. Lipid peroxidation caused by chinoform-ferric chelate in cultured neural retinal cells. Experientia 4, 1561–1563.
Yassin, M.S., Ekblom, J., Xilinas, M., Gottfries, C.G., Oreland, L., 2000. Changes in uptake of vitamin B12 and trace minerals in brains of mice treated with clioquinol. J. Neurol. Sci. 173, 40–44.

〈原　著〉

大腿骨頸部骨折に関連する神経症状の検討
—29年間のSMON検診における縦断的研究—

小長谷正明[1)]　久留　聡[1)]　小長谷陽子[2)]

要　約　**目的**：多彩な神経症状を示す亜急性脊髄視神経ニューロパチー（SMON）患者の大腿骨頸部骨折の頻度を検討するとともに，大腿骨頸部骨折惹起の危険因子となる神経症状を明らかにする．**方法**：対象は「スモンに関する調査研究班」による1979～2007年検診受診患者3,269人，のべ24,187回分の検診票より，大腿骨頸部骨折症例を抽出した．臨床症状の検討は，全大腿骨頸部骨折患者のうち，骨折前2年以内に検診受診者は80例であり，この大腿骨頸部骨折群と，年齢・性・罹病期間をマッチした大腿骨頸部骨折を起こさなかったSMON患者160例を対照群とした．検討した臨床症状は，視力，歩行能力，起立位，下肢振動覚，下肢筋力，下肢痙縮，下肢触覚，下肢痛覚，異常知覚である．また，日常生活動作能力指標であるBarthel Index得点を比較した．**結果**：大腿骨頸部骨折は208人，全検診受診者の6.4%に230回みられ，男女比は21：187であった．年齢階層別の1万人あたりの年間発生件数は，女性では50歳代：7.74件（日本人女性全体は2.41件）と60歳代：18.5件（9.11件）で，それぞれ日本人全体と比較して，有意に頻度が高かった（いずれも$p<0.002$）．男性は40歳以下：2.34件（日本人男性全体は0.3件）（$p<0.02$）と50歳代で8.80件（1.82件）（$p<0.002$）で日本人男性全体より有意に高く，80歳代：8.85件（58.6件）（$p<0.02$）では，有意に低かった．臨床症状の検討では，大腿骨骨折群は対照群と比較して，歩行障害では杖歩行が多く（$p<0.05$），下肢振動覚の高度障害が多かった（$p<0.025$）．他の神経症状の重症度の比率やBarthel Index得点は両群間に差はなかった．**結論**：多彩なSMONの神経症状のうち，振動覚障害，すなわち深部感覚障害に根ざす歩行障害の患者に大腿骨頸部骨折が多かった．深部覚障害が認められる人は，転倒に注意すると同時に，医学的な原因追及や治療が必要である．

Key words：スモン，転倒，大腿骨頸部骨折，深部覚障害，歩行障害

（日老医誌　2010；47：445-451）

はじめに

高齢者や神経疾患患者では，大腿骨頸部骨折は日常生活機能の低下をもたらす主要原因であり，側方や後側方への転倒によって惹起されることが多い[1)]．転倒をきたす要因としては，下肢や体幹の筋力，筋緊張，体幹平衡機能，姿勢調節反射能力の低下などが考えられるが，大腿骨頸部骨折患者でのこれらの神経症状を検討した報告は乏しい．一方，亜急性脊髄視神経ニューロパチー（subacute myelo optico neuropathy：以下SMON）は，整腸剤clioquinol（chinoform，キノホルム）の副作用による神経障害であるが[2)3)]，視覚障害や，深部覚障害による平衡障害，下肢筋力低下など，様々な身体的要因によって歩行が不安定になり，転倒しやすい疾患である．これらの障害は，程度に個人差はあるものの，高齢者一般に単独であるいは複合して存在している．そこで，SMON患者の中で大腿骨骨折をきたした群と，きたさなかった群の臨床症状を比較し，大腿骨頸部骨折に関与する神経症状を明らかにすることは，高齢者の転倒予防にも寄与できると考えられる．

SMONは1950年代と60年代に日本各地で多発し，腹部症状が前駆して，視覚障害と下肢の運動麻痺と感覚障害，自律神経障害が急性に発症した疾患である[2)3)]．当初は感染症や自己免疫疾患が疑われたが，1970年にclioquinolとの因果関係が示され，同剤の使用禁止によって新規患者が発生しなくなり，薬害であることが明らかになった[4)]．また，clioquinolを投与した動物実験において，SMONが再現され，同剤の神経毒性が明らかになった[5)]．日本政府の厚生省およびそれを引き継いだ厚生労働省に

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表1　SMON患者における大腿骨頸部骨折群と対照群の特性

	大腿骨頸部骨折群	対照群
人数（人）	80	160
男性：女性	7：73	14：146
SMON発症年齢（歳）	46.1±9.2*	46.2±9.4*
大腿骨頸部骨折発症年齢（歳）	77.6±9.0*	
大腿骨頸部骨折発症時のSMON罹病期間（年）	31.4±6.2*	
調査時年齢（歳）	75.7±8.8*	76.5±10.4*
調査時SMON罹病期間（年）	30.5±5.5*	30.3±8.1*

*：mean±SD
両群間に，SMON発症年齢，調査時年齢，調査時罹病期間に有意差はなかった．

よる「スモンに関する調査研究班」は，SMONが薬害である点を重視し，恒久的対策の一環として，患者の検診を継続的に行ってきた．その結果では，発症から30年以上を経過しても，歩行困難や感覚異常を呈する患者が多数みられ，後遺症が深刻な問題となっている[6][7]．

今回，これまでの検診受診者全員を対象に，SMON患者における大腿骨頸部骨折の発生頻度と，大腿骨頸部骨折患者における臨床症状の関連性を検討したので報告する．

方　法

対象は「スモンに関する調査研究班」による検診を受診した患者3,269人であり，全受診者が統計解析に同意した．1979～2007年に亘る延べ24,187回分の検診票より，股関節骨折あるいは大腿骨頸部骨折と記載されている症例を抽出した．全大腿骨頸部骨折患者のうち，骨折前2年以内に検診を受けた患者は80例であり，臨床症状の検討はこの80例を大腿骨頸部骨折群とし，大腿骨頸部骨折を起こさなかったSMON患者の中から骨折した各症例ごとに年齢・性・罹病期間が一致した2例を抽出した計160例からなる対照群として，両群間で行った（表1）．SMON発症年齢，調査時年齢，調査時SMON罹病期間に関して，両群間に有意差はなかった．

SMON患者における大腿骨頸部骨折の，性別，年齢階層別の1万人あたり年間発生頻度を，2002年における日本人全体での推計値[8]と比較検討した．統計的解析は母比率の検定で行った[9]．

次に，臨床症状として，視力，歩行能力，起立位，下肢振動覚，下肢筋力，下肢痙縮，下肢触覚，下肢痛覚，異常知覚の程度を検討した．また，日常生活動作能力はBarthel Index[10]の得点で評価した．これらの症状の判定や評価は，原則として神経内科医が行った．

視力の障害程度は，全盲，指数弁以下の高度低下，新聞見出し判読可能の中等度低下，およびほぼ正常の4段階に分類した．歩行は不能，介助歩行，杖歩行，不安定独歩，およびほぼ正常の5段階に，起立位は，起立不能，介助，開脚，閉脚および継ぎ足起立の5段階に分類した．下肢振動覚，下肢筋力，下肢痙縮，異常知覚はそれぞれ高度，中等度，軽度，なしの4段階に，下肢触覚と痛覚はそれぞれ，高度低下，中等度低下，軽度低下，過敏，正常の5段階に分類した．大腿骨頸部骨折群と対照群との間での，それぞれの臨床症状の障害度の比率の差を，Kolmogorov-Sminorff test[11]によって検定した．両群間のBarthel Index平均得点の差の検討は，Student's t-test[12]で行った．

結　果

検診を受けた患者3,269人のうちで，208人（6.4%）に大腿骨頸部骨折がみられ，男女比は21：187であった．22人が2回の大腿骨頸部骨折をしており，骨折回数は，延べ230回であった．

初回骨折の年齢階層別度数は，40歳以下では，2件（男性：女性＝2：0），40歳代では，3件（0：3），50歳代では25件（7：18），60歳代では41件（3：38），70歳代では56件（6：50），80歳代では54件（1：53），90歳以上では12件（2：10）であり，年齢不明は15件（0：15）であった（図1）．

年齢階層別の1万人あたりの年間骨折発生件数を算出すると，以下の如くであった．女性では40歳代で1.26件（2002年調査における日本人女性の年間発生件数0.58件），50歳代では7.74件（2.41件），60歳代では18.5件（9.11件），70歳代では36.36件（41.07件），80歳代では113.36件（156.10件），90歳以上では226.50件（315.20件）であった（図2）．SMON女性患者と，日本人女性全体との間では，1万人あたりの年間発生比率に，50歳代と60歳代でそれぞれ有意な差が見られた（いずれも$p<0.002$）．

男性では40歳以下で2.34件（2002年調査における日

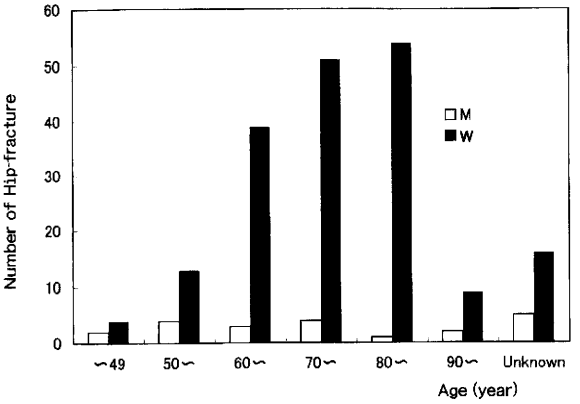

図1　SMON検診受診者における年齢階層別の初回大腿骨頸部骨折発生件数
M：男性；W：女性.

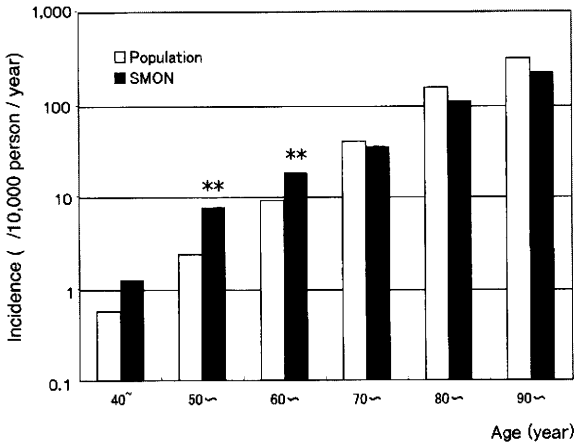

図2　日本人女性全体とSMON女性患者の年齢階層別人口1万人あたり大腿骨頸部骨折年間発生頻度
白：日本人女性全体；黒：SMON女性患者．＊＊：p＜0.002.

本人男性の年間発生件数0.30件)，40歳代では0件(0.44件)，50歳代では8.80件（1.82件)，60歳代では4.44件(5.26件)，70歳代では16.63件（17.49件)，80歳代では8.85件（58.60件)，90歳以上では227.28件（141.39件）であった（図3)．SMON男性患者と，日本人男性全体との間では，40歳以下(p<0.02)，50歳代(p<0.002)および80歳代（p<0.02）で，1万人あたりの年間発生比率に，それぞれ有意な差がみとめられた.

大腿骨頸部骨折群と対照群の各神経症状の障害程度の比率を表2に示す．視力の障害程度の比率に両群間の差はなかった．歩行障害の程度の比率に関しては，杖歩行のみで大腿骨頸部骨折群（43.8%）と対照群（28.1%）との間で有意差が認められた（p<0.05)．下肢振動覚障害

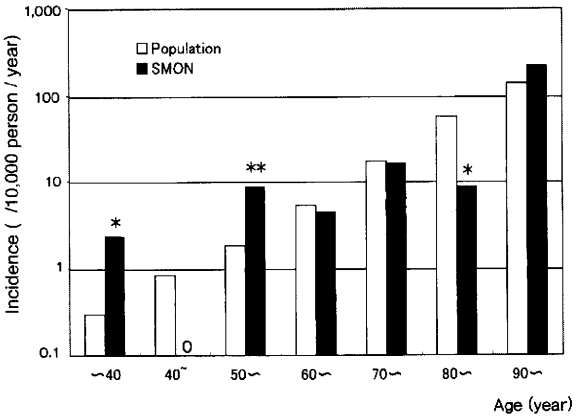

図3　日本人男性全体とSMON男性患者の年齢階層別人口1万人あたり大腿骨頸部骨折年間発生頻度
白：日本人男性全体；黒：SMON男性患者．＊：p＜0.02；＊＊：p＜0.002.

の程度の比率については，高度障害のみで大腿骨頸部骨折群（51.9%）と対照群（32.0%）の間に有意差がみられた（p<0.025)．しかし，起立位，下肢筋力，下肢痙縮，触覚，痛覚，異常知覚の各神経症状の障害程度の比率には，両群間に有意な差はなかった.

Barthel Indexの平均得点は，評価が可能であった大腿骨頸部骨折群70人では，80.63±21.26（mean±SD）点，140人の対照群では84.51±17.83点であり，両群間に有意差はなかった.

考　察

我々は，スモン患者124例の大腿骨頸部骨折を検討し，女性では比較的若年に多いことをすでに示したが[13]，今回，検索期間を拡大するとともに精度を高めて再調査し，さらに骨折前の臨床症状との関係を明らかにした.

日本人全体の大腿骨頸部骨折の年間発生頻度は加齢とともに著しく増加しており[8]，片対数でグラフを描くとほぼ直線状となり，これは指数関数状の発生増加を示している．性差に関しては，40歳代までは男性の方が高頻度であるが，50歳代からは逆転して女性に増え，70歳代以降は女性が男性の2～2.5倍の頻度で高い．比較的若年の年齢層で，男性の方が高頻度に大腿骨頸部骨折が発生するのは，肉体的活動量が高いためと考えられる．高齢になるに従って大腿骨頸部骨折が女性に圧倒的に多くなるのは，閉経後の骨粗鬆症や大腿骨頸部が側方に突出しているなどの解剖学的特徴によると考えられる.

今回の検討では，SMONにおける大腿骨頸部骨折は，全検診受診者の6.4%，208人にのべ230回みられた．女性の数は男性の約9倍であったが，高齢者一般におけ

表2　大腿骨頸部骨折群と対照群の神経症状重症度の割合の比較

A. 視力（NS）

	N	全盲	指数弁	中等度低下	ほぼ正常
骨折群：人（%）	79	1（1.3）	4（5.1）	35（44.3）	39（49.4）
対照群：人（%）	159	1（0.6）	6（3.8）	66（41.5）	86（54.1）

B. 歩行（$p < 0.05$）

	N	不能	介助歩行	杖歩行	不安定独歩	正常
骨折群：人（%）	80	7（8.8）	11（13.8）	35（43.8）	24（30.0）	3（3.8）
対照群：人（%）	160	13（8.1）	20（12.5）	45（28.1）	74（46.3）	8（5.0）

C. 起立位（NS）

	N	不能	介助	開脚	閉脚	継ぎ足
骨折群：人（%）	78	7（9.0）	17（21.8）	28（35.9）	22（28.2）	4（5.1）
対照群：人（%）	160	8（5.0）	31（19.4）	50（31.3）	57（35.6）	14（8.8）

D. 下肢振動覚障害（$p < 0.025$）

	N	高度	中等度	軽度	なし
骨折群：人（%）	79	41（51.9）	27（34.2）	9（11.4）	2（2.5）
対照群：人（%）	153	49（32.0）	71（46.4）	32（20.9）	1（0.7）

E. 下肢筋力低下（NS）

	N	高度	中等度	軽度	なし
骨折群：人（%）	80	9（11.3）	29（36.3）	34（42.5）	8（10.0）
対照群：人（%）	158	17（10.8）	41（25.9）	76（48.1）	24（15.2）

F. 下肢痙縮（NS）

	N	高度	中等度	軽度	なし
骨折群：人（%）	80	2（2.5）	17（21.3）	23（28.8）	38（47.5）
対照群：人（%）	159	7（4.4）	26（16.4）	47（29.6）	79（49.7）

G. 下肢触覚障害（NS）

	N	高度	中等度	軽度	過敏	なし
骨折群：人（%）	80	8（10.0）	42（52.5）	17（21.3）	8（10.0）	5（6.3）
対照群：人（%）	158	15（9.5）	72（45.6）	47（29.7）	18（11.4）	6（3.8）

H. 下肢痛覚障害（NS）

	N	高度	中等度	軽度	過敏	なし
骨折群：人（%）	80	7（8.8）	37（46.3）	12（15.0）	22（27.5）	2（2.5）
対照群：人（%）	158	14（8.9）	63（39.9）	40（25.3）	37（23.4）	4（2.5）

I. 異常知覚（NS）

	N	高度	中等度	軽度	なし
骨折群：人（%）	80	22（27.5）	45（56.3）	8（10.0）	5（6.3）
対照群：人（%）	157	35（22.3）	96（61.1）	23（14.6）	3（1.9）

Kolmogorov-Sminorff test, NS：有意差なし

る大腿骨頸部骨折頻度の性差と，女性のSMON患者数が男性の約3倍であることなどを考え合わせると，この発生件数の性差はほぼ妥当であると考えられる．

また，SMON患者では男女とも60歳代以下で，日本人一般に較べて大腿骨頸部骨折の頻度が高い傾向がみられた．SMON患者は元来歩行障害があるものの比較的若い年齢層では行動量が多く，転倒などによる大腿骨頸部骨折が起こり易い．一方，高齢になるとSMON患者

の行動量は減少し，また，長期間に亘る身体障害により易転倒性の認識が強くて用心深くなり，転倒自己効率感が高くないことにより[14]，一般人と同程度の，あるいは80歳代男性のように低い頻度になると推定される．また，一般的に認知症も大腿骨頸部骨折の危険因子とされているが，SMONは認知機能低下をきたさない疾患であることも[15]，高齢患者においても，身体障害の割に大腿骨頸部骨折の頻度が高くない理由と考えられる．

水落ら[16]によれば，大腿骨骨折はスモン患者の転倒による骨折全体の約10%であり，軽視できない頻度である．美和ら[17]の44名のスモン患者の転倒調査では，84%が検診前3カ月以内に転倒を経験しており，転倒場所としては室内が56%と，室外の38%に較べて多く，在宅高齢者の転倒の70%が屋外であるのと様相が異なっている．転倒場面としては歩行時が全転倒件数の約42%，方向転換時が約22%．起き上がる時が約16%となっており，重心移動が転倒につながっていると指摘している．

なお，本研究では，大腿骨頸部骨折は歩行不能患者においても起きているが，これは床上介護や移乗の際の事故によると推定される．

SMONの長期経過例の臨床症状は，下肢の表在覚障害と異常知覚，深部覚障害，脱力と痙縮が典型的であり，視力障害は回復する例が多いが，高齢になると白内障が高頻度となる[6][7]．痙縮以外のこれらの症状はいずれも転倒の危険因子とされている[18]．視力障害に関しては，今回のSMON患者での検討結果では，大腿骨頸部骨折群で明らかに視力障害が強いとはいえなかった．

SMONは病理学的には感覚伝導路である脊髄後索の変性が強く，運動伝導路である側索の変性は頸髄では軽微だが[19]，腰髄では強いといわれている[5]．これらの障害の結果として，起立障害や様々な程度の歩行障害がもたらされ，転倒しやすくなる．大腿骨頸部骨折をきたしたSMON患者の歩行能力を見ると，杖歩行の比率が高く，ある程度の歩行能力が残存している人が多かった．しかし，対照群と較べると，必ずしも下肢の筋力が低下している患者の割合が多くはなかった．一方，感覚障害のうち，高度の振動覚障害の割合が骨折群で高かったことが特徴的であった．振動覚は深部覚の一種であり，直接には姿勢維持機能に関わらないが，これが障害されていることは臨床的には他の深部覚障害の存在を推定する徴候であり，高度障害は下肢や体幹のバランスが悪いことを伺わせる．一般の高齢女性においても，転倒を複数回きたした群では振動覚障害が強いことが報告されている[20]．

姿勢維持に関わる感覚としては，内耳の三半規管による平衡感覚，視覚，さらに関節の位置覚や筋肉の運動覚などの深部感覚である．高度の深部覚障害では，四肢や体幹の位置，筋肉の運動状況や張力を平衡中枢である小脳に入力できず，体幹のバランスが損なわれやすい．日常臨床で起立位の被検者に閉眼させるロンベルグ試験では，視覚情報による補正ができなくなり，深部覚障害がある場合は体幹動揺が大きくなる．生活場面では不安定な動揺性の歩行となり，姿勢調節反射も損なわれるために，バランスを崩したときに有効なステップが踏めずに転倒しやすくなる．側方や斜め後方へ転倒した場合は大腿骨頸部に衝撃が加わり，同部の骨折をきたすと考えられる[1]．

神経疾患では転倒はしばしばみられ，パーキンソン病，多発性ニューロパチー，および脊髄障害に多く，歩行や平衡感覚に関与する臨床症状との関連性が見られている[21]．パーキンソン病では同年齢の人に比べて骨折のリスクが2倍も高く，とりわけ大腿骨骨折が多いことが報告されている[22]．また，パーキンソニズムと小脳失調をきたす多系統萎縮症では，平衡障害が強い小脳型で，転倒リスクが高いとされている[23]．末梢神経障害では比較的歩行能力がある人が転倒しやすく，深部覚障害と転倒の間に有意な相関がみられている[24][25]．Menz[25]によれば糖尿病性ニューロパチーでは，歩行バランスの悪化要因としては，振動覚や触覚障害の方が，視覚障害や筋力低下より影響が大きいという．これらの報告は，今回の検討で明らかにした，大腿骨頸部骨折をきたしたSMON患者に，高度の下肢振動覚障害が多かったことと一致している．すなわち，下肢筋力低下などの症状に加えて，深部覚性の運動失調のために歩行障害が強いSMON患者が，より転倒しやすく，大腿骨頸部骨折をきたしやすいと考えられる．

また，SMONにおける大腿骨頸部骨折への骨粗鬆症の関与も考慮しなければならない．SMONでは同年齢層の健常者と比較して骨密度が低く，その原因としては，消化管障害によるカルシウムやビタミンDなどの吸収障害説や，単なる運動量減少による骨の廃用萎縮説などがある．SMON患者の骨塩量は同年齢層の健常者と比較して距骨では低下しているが[27]，腰椎の椎骨では変化がないことが報告されており[28]，下肢の運動障害の結果の可能性が考えられる．しかし，スモン調査研究班による検診では，組織的には骨密度や骨代謝関連指標，内分泌的検査などは行ってきておらず，SMON患者の中における大腿骨頸部骨折群と対照群との差を明らかにすることは出来なかった．

以上のように，大腿骨頸部骨折をきたす神経機能の要

因を，多彩な神経障害を示すSMONの多数例で検討した結果，深部感覚障害による歩行・起立障害が主要な原因の一つとして考えられた．高齢者の転倒に関連した危険因子としては，視力障害よりも深部覚障害を反映するロンベルグ率が高いことが報告されている[18]．さらに高齢者では深部知覚の低下や，これを伝える末梢神経の大径有髄線維の脱落が起っている[29]とされており，今回の検討結果と考え合わせると，深部感覚の高度障害を示す患者や高齢者は，大腿骨頸部骨折の危険がより高いといえる．易転倒性のある人には，深部感覚障害をもたらす末梢神経障害や，脊髄後索を圧迫する脊椎疾患などの有無を検索し，治療やリハビリテーションを行う必要がある．

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文　献

1) 島　浩人，依岡　徹，吉本和徳，神先秀人，坪山直生，中村孝志：大腿骨頸部骨折を起こし易い転倒方向について―転倒装置を用いた分析．Osteoporosis Japan　2004; 12: 384–402.
2) Sobue I, Ando K, Iida M, Takayanagi T, Yamamura Y, Matsuoka Y: Neuromyelopathy with abdominal disorders in Japan. A clinical study of 752 cases. Neurology 1971; 21: 168–173.
3) Sobue I: Clinical aspects of subacute myelo-optico-neuropathy (SMON), In: Intoxications of the nervous system part 2. Handbook of clinical neurology, vol 37, Vinken PJ, Bruyn GW, Cohen MM (eds), North-Holland, Amsterdam, 1979, p115–139.
4) Tsubaki T, Honma Y, Hoshi M: Neurological syndrome associated with clioquinol. Lancet 1971; 1: 696–697.
5) Shiraki H: Neuropathological aspects of the etiopathogenesis of subacute myelo-optico-neuropathy (SMON), In: Intoxications of the nervous system part 2. Handbook of clinical neurology, vol 37, Vinken PJ, Bruyn GW, Cohen MM (eds), North-Holland, Amsterdam, 1979, p141–198.
6) Konagaya M, Matsumoto A, Takase S, Mizutani T, Sobue G, Konishi T, et al.: Clinical analysis of subacute myelo-optico-neuropathy: sequelae of clioquinol at 32 years after its ban. J Neurol Sci 2004; 218: 83–90.
7) 小長谷正明：全国スモン検診の総括．神経内科　2005; 63: 141–148.
8) 折茂　肇，坂田清美：第4回大腿骨頸部骨折全国頻度調査成績―2002年における新発生患者数の推定と15年間の推移．日本医事新報　2004; 4180: 25–30.
9) 丹後俊郎：母比率pの検定と信頼区間．新版医学への統計学（古川俊之監），朝倉書店，東京，1993，p113–115.
10) Mahoney FI, Barthel DW: Functional evaluation: The Barthel Index. Maryland State Med J 1965; 14: 61–65.
11) 菅　民郎：コルモゴロフ・スミノフの検定（2標本）．改訂版［EXCEL統計］のための統計分析の本，エスミ，東京，2001，p233–237.
12) 市原清志：2標本t検定．バイオサイエンスの統計学，南江堂，東京，1990，p72–87.
13) 小長谷正明，松岡幸彦，氏平高敏：スモンにおける大腿骨頸部骨折の検討．神経内科　2005; 62: 477–480.
14) 加藤智香子，猪田邦雄，原田　敦：介護老人保健施設の女性高齢者における日常生活活動と乖離した高い転倒自己効率感が転倒発生に与える影響．日本老年学会誌　2009; 46: 428–435.
15) 大槻美佳，松本昭久，森若文雄，田代邦雄：スモン患者における高次機能と加齢の関連．神経内科　2005; 63: 157–161.
16) 水落和也，菊池尚久，長谷川一子：スモンの障害予防に関する研究：転倒が運動障害に及ぼす影響．厚生労働科学研究費補助金（難治性疾患克服研究事業）スモンに関する調査研究班平成17年度総括・分担研究報告書，2006，p106–108.
17) 美和千尋，杉村公也，清水英樹，伊東恵美，森　明子，寶珠山稔：スモン患者の転倒調査．綜合リハ　2006; 34: 688–692.
18) 石崎久義，Ilmari　P：高齢者の姿勢制御機構　転倒と視覚の関係について．Equilibrium Research　1995; 54: 40.
19) 今野秀彦，高瀬貞夫，福井俊彦：スモン長期症例における病理像―全経過28年の剖検例から．脳と神経　2001; 53: 875–880.
20) Lord SR, Ward JA, Williams P, Anstey KJ: Physiological factors associated with falls in older community-dwelling women. J Am Geriatr Soc 1994; 42: 1110–1117.
21) Stolze H, Klebe S, Zechlin C, Baecker C, Friege L, Deuschl G: Falls in frequent neurological diseases. Prevalence, risk factors and aetiology. J Neurol 2004; 251: 79–84.
22) Genever RW, Downes TW, Medcalf P: Fracture rates in Parkinson's disease compared with age- and gender-matched controls: a retrospective cohort study. Age and Ageing 2005; 34: 21–24.
23) 橋口修二，乾　俊夫：神経系疾患と転倒・転落―多系統萎縮症における転倒・転落の特徴．医療　2006; 60: 33–36.
24) 岡　伸幸，杉山　博，川崎照晃，水谷江太郎，松井　大：ニューロパチーにおける転倒．臨床神経学　2005; 45: 207–210.
25) Cavanagh PR, Derr JA, Ulbrechr JS, Maser RE, Orchard TJ: Problems with gait and posture in neuropathic patients with insulin-dependent diabetic mellitus. Diabetic Med 1992; 9: 469–474.
26) Menz HB, Lord SP, St George R, Fitzpatrick RC: Walking stability and sensorimotor function in older people with diabetic peripheral neuropathy. Arch Phys Med Rehabil 2004; 85: 245–252.
27) Yamanaka K, Fujiwara N：Factor related ultrasonic bone density in subacute myelo-optico neuropathy patients. 名古屋学芸大学健康・栄養研究所年報　1号，

2007, p19–25.
28) 小西哲郎, 小澤恭子, 小牟礼修, 松井　真, 岩村京子, 西田祐子ほか：スモン患者の腰椎骨密度について. 厚生省特定疾患スモン調査研究班平成10年度研究報告書, 1999, p173–175.
29) 東儀英夫, 塚越　広, 豊倉康夫：末梢神経の退行性変化. 神経研究の進歩　1973; 17: 679–690.

Evaluation of neurological symptoms related to hip fracture in a 29-year longitudinal study of subacute myelo-optic-neuropathy (SMON)

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Abstract

Aim: Hip fracture in elderly people is a major risk factor in the deterioration of activities of daily living (ADL). The aim of this study was to investigate the incidence of hip fractures and the neurological symptoms contributing to hip fracture in patients with subacute myelo-optic-neuropathy (SMON), a drug-induced neurological disease manifesting various symptoms.

Methods: We investigated the incidence of hip fracture in 3,269 SMON patients with 24,187 medical check-ups from 1979 through 2007 by the SMON Research Committee in Japan. Neurological symptoms were evaluated in 80 patients who had undergone clinical examinations within 2 years before the fracture (hip-fracture group: age at examination = 75.7 ± 8.8 years (mean ± SD)), and the control group (160 SMON patients without a history of hip fracture; 76.5 ± 10.4) were matched for age, gender, and duration of illness. Incidence of hip fracture in SMON as well as severity of visual acuity, motor and sensory symptoms, and ADL were investigated.

Results: A total 230 hip fractures occurred in 208 patients (6.4%) with a men-to-women ratio of 21 : 187. In comparison with the Japanese general population, SMON patients showed a statistically high incidence of hip fracture in the 50s and 60s age groups in women ($p < 0.002$ in both), and in those under 40 ($p < 0.02$) and in their 50s ($p < 0.002$) in men. In those with neurological symptoms related to gait, the percentage of subjects who could walk with crutches was significantly higher in the hip-fracture group (43.8%) than in the control group (28.1%) ($p < 0.05$). Analysis of the vibratory sensation revealed that the hip-fracture group showed a significantly higher percentage of severe impairment (51.9%) than the control group (32.0%) ($p < 0.025$). There were no significant differences in variance between the two groups in other clinical symptoms or ADL.

Conclusions: Impairment of vibration sense, a deep sensation, is more likely to be associated with falling and hip fracture than visual acuity or other neurological symptoms in SMON patients. Those persons with vibration sense disturbance, such as elderly or patients with neurological diseases, should be particularly cautious of falling.

Key words: *Subacute myelo-optic-neuropathy, Falls, Femoral neck fracture, Deep sensation, Gait disturbance*
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Current Perception Threshold in Subacute Myelo-Optico-Neuropathy

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ABSTRACT

We report the first current perception threshold (CPT) examination of sensory disturbance in subacute myelo-optico-neuropathy (SMON). SMON patients experience serious neurological symptoms, including dysesthesia, sensory loss, motor weakness, and visual impairment. During CPT examination, 5 Hz, 250 Hz, and 2,000 Hz stimulations were used to stimulate C fibers, A-δ fibers, and A-β fibers, respectively. Ten SMON patients (mean age, 73.8 ± 8.4 years) and ten age-matched controls (72.3 ± 6.3 years) were studied using CPT measured at the index finger and near the external malleolus. The CPTs to 250 Hz and 2,000 Hz stimulations near the external malleolus were significantly higher and the CPT to 5 Hz stimulation was significantly lower in the SMON group than in the control group. Although peripheral nerve impairment is mild in SMON, pathological examination shows a decrease of large fibers. This is thought to increase the CPTs to 250 Hz and 2,000 Hz stimulations. The center of the gate control of pain exists in the posterior horn receiving information from the dorsal root ganglion. The dorsal root ganglion at the lumber cord is strongly impaired in SMON; therefore, the gate control may not work effectively, and decreases CPT to 5 Hz stimulation.

KEYWORDS: C fiber, clioquinol, current perception threshold, drug-induced disease, sensory disturbance, subacute myelo-optico-neuropathy

INTRODUCTION

Subacute myelo-optico-neuropathy (SMON) is a disease that causes visual impairment, and sensory disturbance and motor weakness in the lower extremities, preceding abdominal symptoms, such as diarrhea or constipation (Shiraki, 1979; Sobue, 1979; Sobue et al., 1971; Tsubaki, Honma, & Hoshi, 1971). It occurred frequently in the 1960's in Japan, and more than 10,000 people were affected. Newly affected SMON patient decreased dramatically in 1970 because clioquinol (5-chloro-7-iodo-8-hydroxyquinolin) usage was suspended by Japanese government. The cause of SMON is an adverse event associated with the use of clioquinol as an antiflatulent, and it became a social problem in Japan. When patients had a daily intake of more than 0.6 g of clioquinol for more than 14 days, the symptoms of SMON appeared within several days (Nakae, Yamamoto, & Igata, 1971). It is a historical disease in Japan; this is considered as the origin of drug-induced disease. Since the discontinuation of the use of clioquinol in 1970, the appearance of newly affected patients stopped. However, there are 2,000 or more patients who are still affected by the sequelae associated with this disease, such as dysesthesia, sensory loss, motor weakness, and visual impairment (Konagaya et al., 2004; Sobue et al., 1971).

Sensory disturbance in SMON usually occurs symmetrically in the lower extremities. Even though tactile sensation and pain sensation are sometimes reduced, hypersensitivity or paresthesia is often present. Numbness is associated with peculiar symptoms, such as feeling an electric shock, tingling ache, something stuck to the sole, and tight ankles (Sobue, 1979). Despite the fact that the patients often experience these symptoms, there are many cases where an objective evaluation is difficult. Even though objective examination is made, abnormal findings rarely proved because routine sensory nerve conduction velocity and somatosensory-evoked potentials reflect A-β fiber

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impairment and do not reflect A-δ and C fiber impairment. Moreover, differentiating these symptoms from the symptoms of other diseases, especially diabetes mellitus or alcoholic neuropathy, is also difficult in some cases.

The current perception threshold (CPT) examination is useful for the evaluation, detection, screening, and diagnosis of peripheral nervous system diseases (Evans et al., 1992; Takekuma, Ando, Niino, & Shimokata, 2000; Weseley, Liebowitz, & Katims, 1989). Stimulations of 5 Hz, 250 Hz, and 2,000 Hz are used to stimulate C fibers, A-δ fibers, and A-β fibers, respectively (Dotson, 1997; Masson & Boulton, 1991; Masson, Veves, Fernando, & Boulton, 1989; Pitei, Watkins, Stevens, & Edmonds, 1994; Takekuma et al., 2000). This study examined SMON's sensory disturbance using the CPT. This is the first CPT examination of sensory disturbance in SMON.

MATERIALS AND METHODS

Ten SMON patients (mean age, 73.8 ± 8.4 years) and ten age-matched healthy controls (mean age, 72.3 ± 6.3 years) participated in this study. Table 1 shows the patients' characteristics. All patients showed numbness, dysesthesia, or pain in the lower extremities, but they had no sensory disturbances in the upper extremities. Written informed consent was obtained from all of the patients who attended the Neurology Clinics of the Nihon University Itabashi Hospital and Surugadai Nihon University Hospital. The patients who also had other neurological diseases, such as cerebral vascular disease, diabetes mellitus, and lumbar spondylosis, were excluded.

The Neurometer CPT/C (Neurotron, Inc., Baltimore, MD, USA) was used to measure CPT. This device delivers sinusoidal electrical stimuli at frequencies of 5 Hz, 250 Hz, and 2,000 Hz. CPT was measured bilaterally at the index finger and near the external malleolus. The patients were asked to identify the presence or absence of the stimulus through a forced choice protocol. After an initial tentative threshold was determined, the patient was given stimuli that varied around the presumed threshold to confirm threshold stability and repeatability. Sham stimulation was given by turning off all current without informing the patients in order to prevent guessing.

The statistical analysis was performed using Mann-Whitney's U test for comparison between the SMON group and the control group.

RESULTS

Table 2 shows a comparison between the SMON group and the control group. The CPT to 2,000 Hz stimulation and the CPT to 250 Hz stimulation near the external malleolus were significantly higher in the SMON group (334 ± 61 CPT unit and 96 ± 37 on the right side, 349 ± 46 and 100 ± 31 on the left side) than in the control group (231 ± 28 and 76 ± 15 on the right side, 235 ± 24 and 78 ± 13 on the left side), whereas the CPT to 5 Hz stimulation was significantly lower in the SMON group (31 ± 9 on the right side, 31 ± 6 on the left side) than in the control group (50 ± 11 on the right side, 52 ± 13 on the left side). No significant differences were observed between the right side and the left side with any stimulation in both groups.

TABLE 1 Clinical features of SMON

Patient	1	2	3	4	5	6	7	8	9	10
Age (years)	81	75	71	60	62	70	72	84	74	78
Sex	F	F	F	F	F	M	M	F	F	M
Duration of disease (years)	40	41	44	40	40	43	38	39	39	39
U/E weakness[a]	0	0	0	0	0	0	0	0	0	0
L/E weakness[a]	0	2	1	1	1	1	0	2	1	0
PTR	→	→	↑	↑	↑	↓	→	↓	↑	→
ATR	→	→	→	↓	↑	↓	→	↓	↑	→
Grade of severity of sensory disturbance[a]	3	2	3	2	1	2	2	2	1	1
Stuck to the sole	+	+			+		+	+	+	+
Tight ankles		+		+	+	+		+	+	
Electric shock		+	+		+	+	+	+		+
Tingling ache	+	+	+			+	+			
Coldness		+		+	+	+	+	+		

[a]: 3, severe; 2, moderate; 1, mild; 0, none.
U/E, upper extremities; L/E, lower extremities; ↑, hyper; ↓, hypo; →, normal.
All patients gave their written informed consent.

TABLE 2 Mean and standard deviation of current perception thresholds (CPT unit)

		5 Hz	250 Hz	2000 Hz
SMON	R hand	76 ± 27	129 ± 37	271 ± 39
	L hand	80 ± 23	133 ± 32	279 ± 37
	R foot	31 ± 9**	96 ± 37*	334 ± 61**
	L foot	31 ± 6**	100 ± 31*	349 ± 46**
Control	R hand	81 ± 19	126 ± 19	286 ± 25
	L hand	86 ± 22	129 ± 18	276 ± 20
	R foot	50 ± 11	76 ± 15	231 ± 28
	L foot	52 ± 13	78 ± 13	235 ± 24

*$p < .05$ compared to the control group.
**$p < .01$ compared to the control group.
Hand, the index finger; foot, near the external malleolus; one CPT unit = 10 μAmp.

In addition, no significant differences were seen in the CPTs to 5 Hz, 250 Hz, and 2,000 Hz stimulations at the index finger between the SMON group and the control group.

DISCUSSION

CPT can be performed noninvasively and easily without distress (Katims, Rouvelas, Sadler, & Weseley, 1989). It is useful to measure the level of peripheral neuropathy. Stimulation at 5 Hz, 250 Hz, and 2,000 Hz is used to stimulate C fibers, A-δ fibers, and A-β fibers, respectively (Dotson, 1997; Egashira & Matsuyama, 1982; Evans et al., 1992; Katims et al., 1989).

The precise biochemical mechanism behind SMON is not fully understood. It is speculated that clioquinol may work like heavy metals, such as iron and zinc (Arbiser et al., 1998), have a pro-oxidant effect (Benvenisti-Zarom, Chen, & Regan, 2005) and disturb the retention of vitamin B_{12} (Yassin, Ekblom, Xilinas, Gottfries, & Oreland, 2000). The neurological findings observed in SMON are thought to be a type of central axonopathy (Shibasaki, Kakigi, Ohnishi, & Kuroiwa, 1982). It is a condition associated with axonal degeneration in the distal portion of the long nerve fibers within the central nervous system. The pathological findings are symmetrical myelin pallor of the lateral and posterior funiculi of the spinal cord, the optic nerve, and peripheral nerves (Konno, Takase, & Fukui, 2001; Shiraki, 1979).

Although the impairment in the peripheral nerves is thought to be mild (Egashira & Matsuyama, 1982), pathological findings show a decrease of large fibers (Tateishi, 2000). This is thought to increase the CPT to 250 Hz and 2,000 Hz. The CPT to 5 Hz is not increased because unmyelinated fibers are preserved. Yamashita et al. (2002) examined CPT in 48 lumbar radiculopathy patients suffering from lumbar disk herniation compared with 11 control subjects. They found CPT in the patient group was significantly higher than those in the control subjects at 2,000 Hz and 250 Hz, while there was no difference at 5 Hz. Also they found CPT to 5 Hz was significantly higher in patients with severe pain than in those with less pain. Their findings do not explain our finding that CPT to 5 Hz was lower in our SMON group than in the control group. It may be due to the difference in the mechanism and/or the distribution of the impairment. SMON patients have myelopathy in addition to polyneuropathy, and decreased CPT to 5 Hz might be related to gate control. For C fiber stimulation, 5 Hz stimulation is the most suitable, but it also stimulates A-β fibers and A-δ fibers because it is the strongest stimulation (Dotson, 1997). Although the effect of firing at 5 Hz is less than that at 250 Hz, A-δ fiber may influence the gate control in 5 Hz CPT examination. The center of the gate control of pain is considered to exist in the posterior horn receiving information from the dorsal root ganglion. The dorsal root ganglion at the lumbar cord is strongly impaired in SMON, and degeneration and depletion of neurons or hyperplasia of interstitial tissue may be observed (Shiraki, 1979). The gate control that decreases the pain sensation by C fibers may not work effectively when A-β fibers are impaired. The CPT to 5 Hz, which is related to C fibers, was lower in SMON, consistent with their significant hyperalgesia.

Diabetic patients and heavy alcohol users may present with paresthesia, such as a burning sensation or pain in both lower extremities, as observed in SMON. The CPT examination at 5 Hz and 2,000 Hz stimulation increases in diabetic (Masson & Boulton, 1991; Masson et al., 1989; Pitei et al., 1994) and alcoholic polyneuropathies (Oishi et al., 2002). This is mainly because the peripheral nerves are injured. In contrast, SMON patients showed increased CPT to 250 Hz and 2,000 Hz stimulations and decreased CPT to 5 Hz stimulation. Therefore, a CPT examination may be useful to differentiate sensory disorders caused by other diseases, such as diabetes mellitus and heavy alcohol usage.

REFERENCES

Arbiser, J. L., Kraeft, S. K., van Leeuwen, R., Hurwitz, S. J., Selig, M., Dickersin, G. R., et al. (1998). Clioquinol-zinc chelate: A candidate causative agent of subacute myelo-optic neuropathy. *Molecular Medicine, 4*(10), 665–670.

Benvenisti-Zarom, L., Chen, J., & Regan, R. F. (2005). The oxidative neurotoxicity of clioquinol. *Neuropharmacology, 49*(5), 687–694.

Dotson, R. M. (1997). Clinical neurophysiology laboratory tests to assess the nociceptive system in humans. *Journal of Clinical Neurophysiology, 14*(1), 32–45.

Egashira, Y., & Matsuyama, H. (1982). Subacute myelo-optico-neuropathy (SMON) in Japan. With special reference to the autopsy cases. *Acta Pathology Japan, 32*(Suppl 1), 101–116.

Evans, E. R., Rendell, M. S., Bartek, J. P., Bamisedun, O., Connor, S., & Giitter, M. (1992). Current perception thresholds in ageing. *Age Ageing, 21*(4), 273–279.

Katims, J. J., Rouvelas, P., Sadler, B. T., & Weseley, S. A. (1989). Current perception threshold. Reproducibility and comparison with nerve conduction in evaluation of carpal tunnel syndrome. *ASAIO Transactions, 35*(3), 280–284.

Konagaya, M., Matsumoto, A., Takase, S., Mizutani, T., Sobue, G., Konishi, T., et al. (2004). Clinical analysis of longstanding subacute myelo-optico-neuropathy: Sequelae of clioquinol at 32 years after its ban. *Journal of the Neurological Sciences, 218*(1–2), 85–90.

Konno, H., Takase, S., & Fukui, T. (2001). Neuropathology of longstanding subacute myelo-optico-neuropathy (SMON): An autopsy case of SMON with duration of 28 years. *No To Shinkei, 53*(9), 875–880.

Masson, E. A., & Boulton, A. J. (1991). The Neurometer: Validation and comparison with conventional tests for diabetic neuropathy. *Diabetic Medicine, 8* (Special No.), S63–S66.

Masson, E. A., Veves, A., Fernando, D., & Boulton, A. J. (1989). Current perception thresholds: A new, quick, and reproducible method for the assessment of peripheral neuropathy in diabetes mellitus. *Diabetologia, 32*(10), 724–728.

Nakae, K., Yamamoto, S., & Igata, A. (1971). Subacute myelo-optico-neuropathy (S.M.O.N.) in Japan. A community survey. *Lancet, 2*(7723), 510–512.

Oishi, M., Mochizuki, Y., Suzuki, Y., Ogawa, K., Naganuma, T., Nishijo, Y., et al. (2002). Current perception threshold and sympathetic skin response in diabetic and alcoholic polyneuropathies. *Internal Medicine, 41*(10), 819–822.

Pitei, D. L., Watkins, P. J., Stevens, M. J., & Edmonds, M. E. (1994). The value of the Neurometer in assessing diabetic neuropathy by measurement of the current perception threshold. *Diabetic Medicine, 11*(9), 872–876.

Shibasaki, H., Kakigi, R., Ohnishi, A., & Kuroiwa, Y. (1982). Peripheral and central nerve conduction in subacute myelo-optico-neuropathy. *Neurology, 32*(10), 1186–1189.

Shiraki, H. (1979). Neuropathological aspects of the etiopathogenesis of subacute myelo-optico-neuropathy (SMON). In P. J. Vinken, G. W. Bruyn, M. M. Cohen, et al. (eds.), *Intoxications of the Nervous System Part 2. H andbook of Clinical Neurology* (pp. 141–198). Amsterdam: North-Holland.

Sobue, I. (1979). Clinical aspects of subacute myelo-optico-neuropathy (SMON). In P. J. Vinken, G. W. Bruyn, M. M. Cohen, et al. (eds.), *Intoxications of the Nervous System Part 2. Handbook of Clinical Neurology* (pp. 115–139). Amsterdam: North-Holland.

Sobue, I., Ando, K., Iida, M., Takayanagi, T., Yamamura, Y., & Matsuoka, Y. (1971). Myeloneuropathy with abdominal disorders in Japan. A clinical study of 752 cases. *Neurology, 21*(2), 168–173.

Takekuma, K., Ando, F., Niino, N., & Shimokata, H. (2000). Age and gender differences in skin sensory threshold assessed by current perception in community-dwelling Japanese. *Journal Epidemiol, 10*(1 Suppl), S33–S38.

Tateishi, J. (2000). Subacute myelo-optico-neuropathy: Clioquinol intoxication in humans and animals. *Neuropathology, 20* (Suppl), S20–S24.

Tsubaki, T., Honma, Y., & Hoshi, M. (1971). Neurological syndrome associated with clioquinol. *Lancet, 1*(7701), 696–697.

Weseley, S. A., Liebowitz, B., & Katims, J. J. (1989). Neuropathy of uremia: Evaluation by nerve conduction velocity versus neurospecific current perception threshold. *Nephron, 52*(4), 317–322.

Yamashita, T., Kanaya, K., Sekine, M., Takebayashi, T., Kawaguchi, S., & Katahira, G. (2002). A quantitative analysis of sensory function in lumbar radiculopathy using current perception threshold testing. *Spine, 27*(14), 1567–1570.

Yassin, M. S., Ekblom, J., Xilinas, M., Gottfries, C. G., & Oreland, L. (2000). Changes in uptake of vitamin B(12) and trace metals in brains of mice treated with clioquinol. *Journal of the Neurological Sciences, 173*(1), 40–44.

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ORIGINAL ARTICLE

Cervical MRI of subacute myelo-optico-neuropathy

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Study design: Case study.
Objectives: Subacute myelo-optico-neuropathy (SMON) is a severe neuro-degenerative disorder caused by poisoning due to over-dose and prolonged oral administration of clioquinol; this disorder was more frequent during 1957–1970. It is characterized by axonal degeneration and gliosis in the cervical gracile fasciculus. Recently, copper-deficient myelo-neuropathies presenting similar symptoms (that is, painful dysesthesia/paresthesia in the lower limbs, ataxia, spastic paraplegia, autonomic disorders and visual impairment) were reported. Magnetic resonance imaging (MRI) of these patients detected T2-weighted hyperintensities in the cervical spinal cord. An unbalanced zinc–copper metabolism was suggested as one of the candidate pathogenesis of clioquinol toxicity because of its metal-chelating ability. The aim of this study was to present MRI findings of old SMON patients and to compare them with those of current copper-deficient myelo-neuropathies.
Setting: Japan.
Methods: We conducted and analyzed cervical and brain MRIs of seven old SMON patients who contracted the disorder during the 1960s. Serum iron, magnesium, copper, zinc and ceruloplasmin levels were also measured.
Results: Cervical T2-weighted MRIs showed mild volume loss and faint hyperintensities in the dorsal columns, which might reflect residual gliosis. Brain fast fluid-attenuated inversion-recovery images and tractography were normal. Current levels of serum copper and zinc were within almost normal ranges.
Conclusion: Although fainter, the abnormal T2 MRI signals we observed were similar to and occurred in the same locations as those reported in copper-deficient myelo-neuropathy patients. We suggest that these findings are useful to study the mechanism of clioquinol toxicity before using it to treat neurodegenerative diseases such as Alzheimer's disease.
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Keywords: cervical dorsal column; copper-deficient myelo-neuropathy; clioquinol; magnetic resonance imaging (MRI); subacute myelo-optic-neuropathy (SMON)

Introduction

Subacute myelo-optico-neuropathy (SMON) is a neuro-degenerative disorder caused by poisoning due to over-dose and prolonged oral administration of clioquinol. This was shown by an epidemiological study in Japan in 1971,[1] and confirmed by a series of animal experiments.[2] It is characterized by symptoms of severe myelo-neuropathy: painful dysesthesia and paresthesia (such as tingling, stinging, fastening, cold, and sticking sensations) initiating in and moving upwards from the feet, loss of sensations, gait disturbance with ataxic and spastic paraplegia, autonomic disorders, and visual impairment, which almost invariably followed a severe abdominal pain (and sometimes led to loss of consciousness and opisthotonus), constipation, and diarrhea.[3–5] In Japan, there are still more than 2500 SMON patients suffering from severe dysesthesia/paresthesia and ataxic paraplegia.[6] Many autopsy case reports confirmed that the characteristic pathological finding of SMON was a 'dying back neuropathy' in the upper cervical gracile fasciculus (the axon terminals of dorsal root neurons) and the lateral funiculus (the long peripheral terminals of the pyramidal tract). In SMON patients who died at an early stage of the disease, active gliosis and axonal degeneration were detected in the gracile fasciculus (Goll fasciculus), and both the lateral funiculus and dorsal root ganglion were also severely affected.[7] In another autopsy case of SMON, performed 42

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years after the onset of the disease, gliosis was observed in an atrophic gracile fasciculus, but not in the lumbar lateral funiculus.[8]

A recent study of patients with copper deficiency related to hyperzincemia,[9,10] called 'human swayback', presenting with symptoms of severe sensory ataxic myelo-optic-neuropathy and urinary incontinence, showed abnormal magnetic resonance imaging (MRI) findings in their cervical spinal cords. Although the mechanism by which clioquinol toxicity developed into severe myelo-optico-neuropathy remains undefined, Kumar[11] and Schaumburg[12] suggested that it might be similar to that involved in the copper deficiency related to hyperzincemia reported in recent studies,[13–16] as some of the hypocupremia patients presented with severe dysesthesia in their lower extremities.

As the peak period of the SMON epidemic in Japan was about 10 years before development of MRI technology, SMON patients were not examined with MRI at that time. Even now, we are not aware of any literature presenting the MRI findings of SMON patients. To compare SMON with the copper-deficient myelo-neuropathies, we took and analyzed cervical and brain MRIs of some of the surviving SMON patients. We also present their clinical laboratory data, including serum copper, zinc and ceruloplasmin levels.

Methods

Seven SMON patients diagnosed by their neurological manifestations and confirmed histories of intake of high/prolonged doses of oral clioquinol (1.2–2.4 g per day, for 4 weeks to 6 months), who were followed up in our hospital, participated in this study (average age 72.6 ± 9.3 years). Patient characteristics are given in Table 1. All seven patients have survived more than 40 years after the onset of their symptoms, and still have the typical symptoms of SMON: severe dysesthesia, paresthesia, loss of sensation in the lower extremities, sensory ataxia, spastic paralysis and/or visual impairments. All of them have normal scores on the Mini-Mental State Examination (28.8 ± 1.6). At the same time, we also measured serum levels of copper, zinc, iron and ceruloplasmin.

The patients underwent MR imaging for the head and cervical regions on a 3-T MRI scanner (Achieva 3.0T; Philips Medical Systems, Best, The Netherlands) using eight-channel head coils. The imaging sequences for the head region included axial spin-echo T1-weighted (repetition time (TR)/echo time (TE)/number of signal intensity acquisition (NSA) 450 ms/10 ms/1, matrix 320 × 320), turbo spin-echo T2-weighted (TR/TE/NSA 4060 ms/80 ms/2, turbo factor 9, matrix 512 × 512), fast fluid-attenuated inversion-recovery (TR/TE/NSA 9000 ms/120 ms/1, inversion time (TI) 2500 ms, turbo factor 15, matrix 352 × 352) and diffusion-tensor images. For diffusion-tensor imaging we used a single-shot, spin-echo, echo-planar technique; the parameters were TR/TE/NSA 8500 ms/95 ms/2, motion-probing gradient in six directions, *b* value 1000 s mm^{-2}, matrix 128 × 128, voxel size 1.8 × 1.8 × 2.0 mm^3 and no intersection gap. The field of view was 23 cm on all conventional MR images. The imaging

Table 1 Demographics, clinical manifestations and laboratory findings of seven old SMON patients

Patient	*Age*	*Sex*	*Disease duration*	*Onset*							*Present*							
				Oral clioquinol administration	*Abdominal pain*	*Visual impairment*	*Dysesthesia/paresthesia*	*Spastic paralysis*	*Sensory ataxia*	*Autonomic dysfunction*	*Barthel index*	*MMSE*	*Fe* ($\mu g\,ml^{-1}$)	*Mg (mg per 100 ml)*	*Cu* ($\mu g\,ml^{-1}$)	*Zn* ($\mu g\,ml^{-1}$)	*Ceruloplasmin (mg per 100 ml)*
1	56	F	42	+	+	–	+	+	+	+	85	30	56	72	1.8	89	31.1
2	69	F	39	+	+	+	+	+	+	–	100	30	76	87	2.3	87	25.9
3	74	F	40	+	+	+	+	+	+	+	90	28	109	118	2.1	91	32.8
4	76	M	40	+	+	+	+	+	+	+	50	28	67	103	2.7	67	31.4
5	77	F	40	+	+	+	+	+	+	+	70	29	113	97	2.2	77	28.1
6	84	M	41	+	+	+	+	+	+	–	85	26	157	78	2.0	77	22.8
7	66	M	40	+	+	–	+	–	+	–	100	30	114	99	2.5	104	25.3

Abbreviations: MMSE, Mini-Mental State Examination; 1–7, patient number; SMON, subacute myelo-optico-neuropathy.

sequences for the cervical region included sagittal and axial spin-echo T1-weighted (TR/TE/NSA 450 ms/10 ms/4, slice thickness 3 mm) and turbo spin-echo T2-weighted images (TR/TE/NSA 3000 ms/90 ms/2, turbo factor 17, slice thickness 3 mm). The diffusion-tensor imaging data were transferred to an offline workstation (Precision 530; Dell, Round Rock, TX, USA); Philips Research Imaging Development Environment (PRIDE) software (Philips Medical Systems) was used for image analysis. Fiber tracking was performed with FiberTracking V4.1 (PRIDE) on the same workstation.

Results

Although we did not have data from their peak disease period, the patients' current serum copper, zinc, iron and ceruloplasmin levels were all within the normal range (Table 1). We first examined T2-weighted and fast fluid-attenuated inversion-recovery images to see whether there were specific signal changes in the cervical dorsal column, where the severest degeneration was observed in previous autopsies of SMON patients. In three of the seven patients, no abnormal findings on the spinal cord were observed. In the other four patients, we observed medium-sized depressions in the cervical dorsal columns, possibly related to volume loss in the gracile fasciculus. A slight T2-weighted hyperintensity on the cervical spinal cord of a 56-year-old female patient is shown in Figure 1. In the other three patients, such abnormal signals were also observed at the same level of the cervical spinal cord, but they were much weaker than in the first case. Three patients (one with T2-weighted hyperintensity and two without any abnormal findings) exhibited mild compression damages with spondylotic changes or disc herniations in their cervical spinal cords. There were no abnormal findings detected on the brain MRIs, except mild brain atrophies or nonspecific ischemic changes that are probably attributable to aging. In MR tractography, the higher cortico-spinal tracts and visual cortex-related fibers were normally traced.

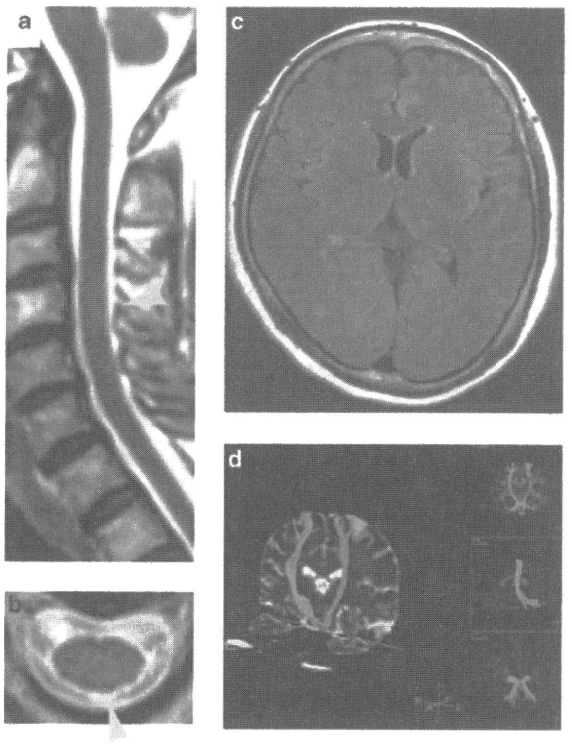

Figure 1 MRI of a 56-year-old female SMON patient. (**a**) T2-weighted image of the cervical spinal cord shows a longitudinal, faint hyperintensity in the sagittal image (arrowhead), (**b**) which was located on the dorsal column of the spinal cord in the axial image (arrowhead). (**c**) Brain MRI FLAIR image appears normal. (**d**) MR tractography shows normal tracing of the pyramidal tract above the brain stem.

Discussion

In this MRI study of old, surviving SMON patients, we found slight hyperintense signals in the cervical spinal cord and medium-sized depressions in their cervical dorsal column. As only old SMON survivors were able to participate in this study, these mild findings might be consistent with a decrease in or cessation of the degeneration followed by gliosis after they had stopped taking clioquinol. On the other hand, the relatively low severity of their symptoms possibly enabled these patients to survive longer, and thus to have a chance for these MRI examinations. Alternatively, such low signals might be considered as an artifact of the 3-T MRI or over-estimation.

The first reported cervical MRI of a copper-deficient myeloneuropathy patient[17] showed a T2-weighted hyperintensity in the dorsal column of the cervical spinal cord (C1–C7). In 11 of 25 clinically diagnosed patients, Kumar *et al.*[18] also found T2-weighted hyperintensities at almost the same portions (central dorsal midline cord involving the dorsal columns) of the spinal cord (spread out from cervical to thoracic, mainly C2–C7 level). Some signals were quite strong and some were faint, which might depend on the severity or the duration of neurological symptoms. In addition, Spinazzi *et al.*[9] presented a patient with copper deciency myelopathy induced by parenteral zinc overloading during chronic hemodialysis with a longitudinal midline central and dorsal lesion in the lower cervical spinal cord. The abnormal T2 hyperintensity signals of these patients disappeared[18] or declined[9,10] as the symptoms recovered when they were treated with copper supplements. The T2 hyperintensities we observed on the cervical spinal cord images of old SMON survivors (4 out of 7) were all much fainter but in the same positions, cervical mid-dorsal columns, and were accompanied by mild volume loss. We considered that these fainter signals might reflect the duration and severity of the clioquinol-induced neuronal damages; the patho-mechanism by which the 'iatrogenic' over-dose and prolonged administration of clioquinol induced SMON may be related to a hyperzincemia-induced copper deficiency, which is described as the most probable theory by Kumar and Knopman[11] and Nations *et al.*[13]

The previously reported hyperintensities seen in T2-weighted MRIs from patients with copper deficiency related to hyperzincemia might partly represent an active phase of SMON.[9] The brain MRIs of all seven SMON patients, as well as the tract-tracing study of their higher cortico-spinal tract and visual cortex-related fibers, were all normal. It was suggested that these portions were quite mildly (or not at all) affected, and had recovered, over a long period, well after they stopped receiving clioquinol. An autopsy record of an SMON patient in our hospital described memory loss, cognitive dysfunction and/or character changes before his death. However, given the lack of brain abnormalities in the current patients, it is difficult to confidently suggest that these psychotic symptoms are a direct result of clioquinol in the central nervous system or other factors related to comorbid metabolic disorders.

Conclusion

Finally, we believe that it is worthwhile to examine MRI evidence in the old SMON patients in order to compare them with those of hyperzincemia-induced copper-deficient myelo-neuropathy patients, which might partly mimic the patho-mechanism of SMON. Even though its toxic mechanism still remains incompletely understood, clioquinol is currently being considered as therapy for other neurodegenerative diseases such as Alzheimer's and Parkinson's diseases in the near future.[19,20] Thus, it is important to further our understanding of the mechanism of clioquinol toxicity before using it therapeutically for neurodegenerative diseases as 'a new therapy'.

Consent

Written informed consent was obtained from all patients for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions: EK was the primary neurologist, conceived the original study, organized and analyzed the data, prepared the draft of the paper, and supervised the entire study. TH contributed to the original idea and helped write and edit the final draft of the paper. SY, YU and YM were consulting neurologists, evaluated MRI data, and assisted with paper editing. TH was the chief consultant regarding neuro-radiological evaluations. MU analyzed the data and helped write and edit the paper.

References

1 Tsubaki T, Honma Y, Hoshi M. Neurological syndrome associated with clioquinol. *Lancet* 1971; **1**: 696–697.
2 Tateishi J, Kuroda S, Ikeda H, Otsuki S. Neurotoxity of iodoxyquinoline: a further study on beagle dogs. *Jpn J Med Sci Biol* 1975; **28**(Suppl): 187–195.
3 Sobue I. Clinical aspects of subacute myelo-optico-neuropathy (SMON). In: Vinken PJ BG, Cohen MM *et al* (eds). *Handbook of clinical neurology*, vol. 37 Elsevier: Amsterdam, 1979. pp 115–139.
4 Shiraki H In: Vinken PJ, Bruyn GW, Cohen MM, *et al.* (eds). *Handbook of Clinical Neurology*. Elsevier: Amsterdam, 1979. pp 141–198.
5 Nakae K, Yamamoto S, Igata A. Subacute myelo-optico-neuropathy (S.M.O.N.) in Japan. A community survey. *Lancet* 1971; 2: 510–512.
6 Konagaya M, Matsumoto A, Takase S, Mizutani T, Sobue G, Konishi T *et al.* Clinical analysis of longstanding subacute myelo-optico-neuropathy: sequelae of clioquinol at 32 years after its ban. *J Neurol Sci* 2004; **218**: 85–90.
7 Shiraki H. Neuropathology of subacute myelo-optico-neuropathy, 'SMON'. *Jpn J Med Sci Biol* 1971; **24**: 217–243.
8 Konno H, Takase S. Neuropathology of longstanding subacute myelo-optico-neuropathy (SMON)]. *Shinkei Naika* 2005; **63**: 162–169.
9 Spinazzi M, De Lazzari F, Tavolato B, Angelini C, Manara R, Armani M. Myelo-optico-neuropathy in copper deficiency occurring after partial gastrectomy. Do small bowel bacterial overgrowth syndrome and occult zinc ingestion tip the balance? *J Neurol* 2007; **254**: 1012–1017.
10 Spain RI, Leist TP, De Sousa EA. When metals compete: a case of copper-deficiency myeloneuropathy and anemia. *Nat Clin Pract Neurol* 2009; 5: 106–111.
11 Kumar N, Knopman DS. SMON, clioquinol, and copper. *Postgrad Med J* 2005; **81**: 227.
12 Schaumburg H, Herskovitz S. Copper deficiency myeloneuropathy: a clue to clioquinol-induced subacute myelo-optic neuropathy? *Neurology* 2008; **71**: 622–623.
13 Nations SP, Boyer PJ, Love LA, Burritt MF, Butz JA, Wolfe GI *et al.* Denture cream: an unusual source of excess zinc, leading to hypocupremia and neurologic disease. *Neurology* 2008; **71**: 639–643.
14 Kumar N, Gross Jr JB, Ahlskog JE. Myelopathy due to copper deficiency. *Neurology* 2003; **61**: 273–274.
15 Hedera P, Fink JK, Bockenstedt PL, Brewer GJ. Myelopolyneuropathy and pancytopenia due to copper deficiency and high zinc levels of unknown origin: further support for existence of a new zinc overload syndrome. *Arch Neurol* 2003; **60**: 1303–1306.
16 Hedera P, Peltier A, Fink JK, Wilcock S, London Z, Brewer GJ. Myelopolyneuropathy and pancytopenia due to copper deficiency and high zinc levels of unknown origin. II. The denture cream is a primary source of excessive zinc. *Neurotoxicology* 2009; **30**: 996–999.
17 Schleper B, Stuerenburg HJ. Copper deficiency-associated myelopathy in a 46-year-old woman. *J Neurol* 2001; **248**: 705–706.
18 Kumar N, Ahlskog JE, Klein CJ, Port JD. Imaging features of copper deficiency myelopathy: a study of 25 cases. *Neuroradiology* 2006; **48**: 78–83.
19 Gouras GK, Beal MF. Metal chelator decreases Alzheimer beta-amyloid plaques. *Neuron* 2001; **30**: 641–642.
20 Kaur D, Yantiri F, Rajagopalan S, Kumar J, Mo JQ, Boonplueang R *et al.* Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity *in vivo*: a novel therapy for Parkinson's disease. *Neuron* 2003; **37**: 899–909.

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