Smad1/5/8 by a protein kinase inhibitor dorsomorphin. The correlation between the levels of phosphorylated nuclear Smad1/5/8 and myogenin indicates a threshold of the Smad signal intensity that is sufficient to maintain myogenic cells in an undifferentiated state (Fig. 6F). When the level of Smad signaling is below the threshold, myogenic progenitor cells begin to undergo terminal myogenic differentiation (Fig. 8B).

Our previous study suggests that the exposure to high concentrations of BMP2 causes extraordinary activation of the Smad signaling pathway resulting in induction of osteogenesis in myogenic cells [9]. Hyper-activated Smad1/5/8 irreversibly prevents myogenesis, whereas spontaneously activated Smad1/5/8 suppresses precocious myogenic differentiation reversibly without exposure to exogenous BMPs. Then, hyperactivation of the Smad signaling pathway results in expression of Smad target genes that are not induced during myogenesis but are required for osteogenesis (Fig. 8B).

Recently, we found that high concentrations of BMPs induce ectopic osteogenesis of Ric10 in a cell density-dependent fashion (Supplementary Fig. S7). The results suggest a continuum in the effect of BMPs between the inhibition of myogenic differentiation and transdifferentiation into an osteogenic cell fate. In addition, low concentrations of BMP2 induced osteogenesis in Ric10 cells at low cell density (Supplementary Figs. S7B and C). Taken together with the Supplementary results, the magnitude of Smad signaling might play a critical role in generation of different fates from myogenic progenitor cells (Fig. 8B). In addition, we have found that the exogenous BMP-induced osteogenesis is facilitated by a co-signal (Yanagisawa and Hashimoto, unpublished). Therefore, cellular context and co-signals may determine whether a given BMP stimulus induces which cell fates. From this point of view, it is very interesting that the migrating Ric10 cells at the margin of a cell mass were refractory when exposed to high concentrations of BMP2 (Supplementary Figs. S7D and E).

Quenching of Smad signaling is rate-limiting for myogenic differentiation

The present study indicates that quenching of the Smad signaling pathway triggers myogenic differentiation under the high cell density culture condition. Serum reduction also lowered the phosphorylation level of the Smad signaling pathway. However, high cell density was more potent for inactivating the Smad signaling pathway than low serum concentration in the medium. The present study shows that the Smad signaling pathway is also rate-limiting for myogenic differentiation induced by serum reduction. However, the enhancement of myogenic differentiation by dorsomorphin was quite limited under the serum-reduced, low cell density culture condition. Thus, it is likely that distinct

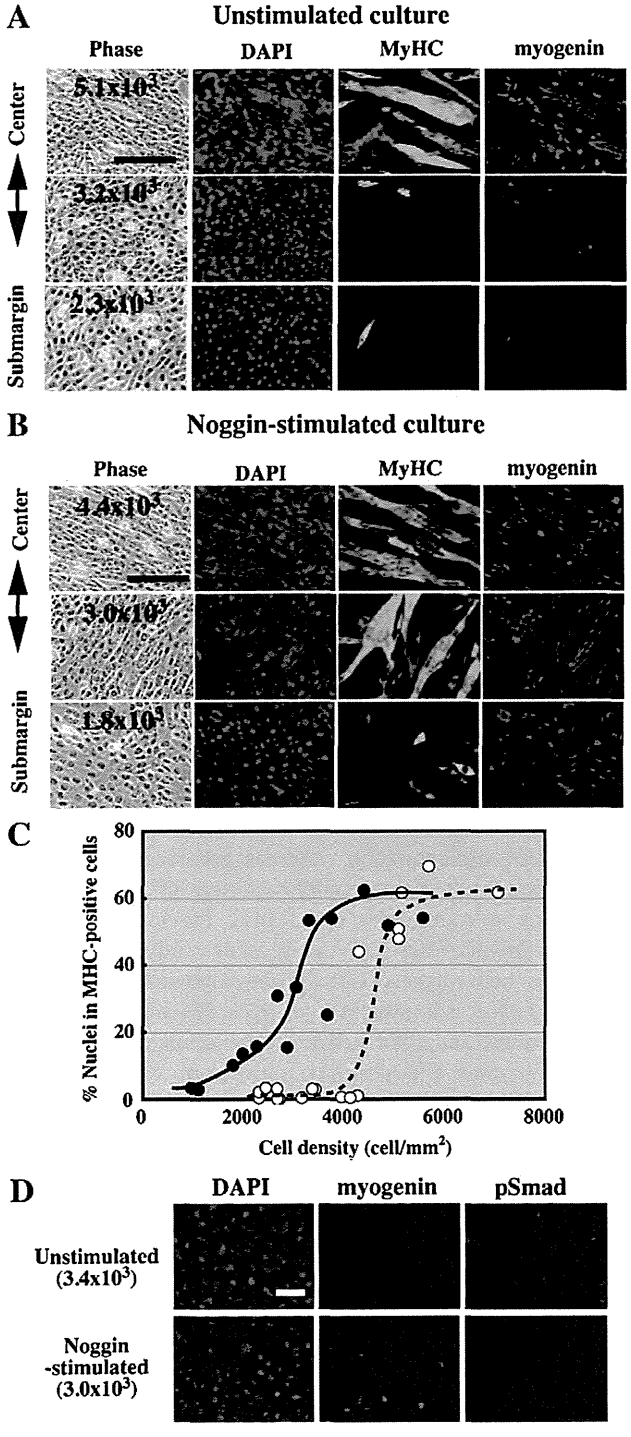

Fig. 7 – Induction of precocious myogenic differentiation by BMP antagonist noggin. (A, B and C) Ric10 cells (5×10^4 cells per 100-μl spot) were cultured in micromasses in pmGM for 24 h and then further cultured in pmGM with (B) or without (A) noggin (5 mg/ml) for up to 24 h. The cells were subjected to immunostaining with anti-myogenin and anti-MyHC antibodies. Nuclei were stained with DAPI. Images of various regions were obtained by phase contrast and epifluorescent microscopy. Numbers in the left-hand panels in A and B represent cell density (cell per mm²). (C) Cell density and the percentages of nuclei in MyHC-positive cells in the total number of nuclei were calculated in cultures stimulated with (solid circles) or without (open circles) noggin. (D) Ric10 cells (5×10^4 cells per 100-μl spot) were cultured in micromasses in pmGM for 24 h and then further cultured in pmGM with (lower panels) or without (upper panels) noggin (5 mg/ml) for up to 24 h. The cells were subjected to immunostaining with anti-myogenin and anti-phosphorylated Smad1/5/8 antibodies. Nuclei were stained with DAPI. Images were obtained by epifluorescent microscopy. The numbers in parentheses at the left of panels represent cell density (cell per mm²) at the end of culture. Scale bars: 50 μm.

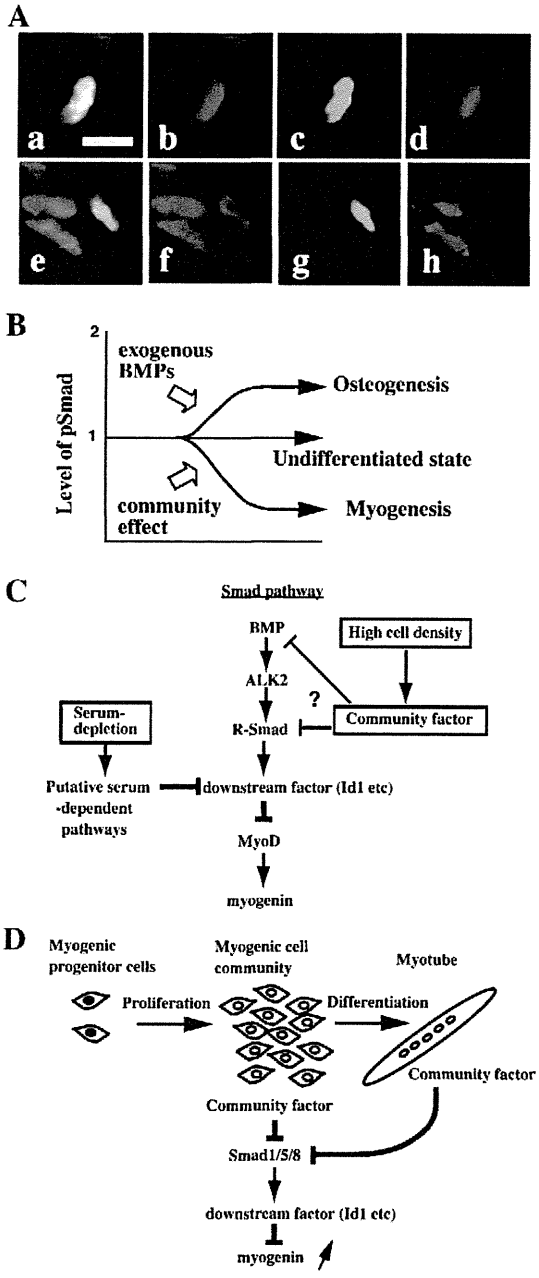

Fig. 8 – Role of Smad signaling pathway in switch between growth and differentiation of postnatal myogenic cells. (A) BPV was injected into the gastrocnemius muscle of rats. Cryosections were prepared from the muscles removed on day 3 (a–d) or 4 (e–h) after BPV injection, and stained with anti-MyoD (c), anti-myogenin (g), anti-phosphorylated Smad1/5/8 (pSmad) (d and h) and DAPI (b and f), respectively. (b, c, and d and f, g, and h) were merged in (a) and (e), respectively. Scale bar: 10 μm. (B) Crucial role of phosphorylated Smad1/5/8 (pSmad) in generation of different fates from myogenic progenitor cells. (C) Hypothetical role of down-regulation of Smad signaling pathway during myogenic differentiation induced in vitro. High cell density and serum reduction synergistically induce myogenic differentiation although they down-regulate downstream factors including Id1 through distinct pathways. (D) Community effect on myogenesis during postnatal muscle growth and repair. See detailed explanation of B, C and D in the "Discussion".

signaling pathways also play a role in the rate-determining step for myogenic differentiation under the serum-reduced, low cell density culture condition. The basal levels of the Smad signaling may induce expression of multiple target genes in undifferentiated, growing myogenic cells. *Id1* is a well-known downstream target gene of the Smad signaling pathway (Fig. 8C). *Id1* encodes an inhibitor protein of the MyoD family and is down-regulated during myogenic differentiation induced by serum reduction [37,39,40]. Previous studies have shown several factors that down-regulate Id1 protein independently of the effects of the BMP–ALK–Smad axis on serum reduction: interferon-inducible protein p204 and micro RNA miR-206 repress Id1 protein and promote myogenic differentiation [39,40]. In addition, lowering the level of p204 inhibits myogenic differentiation in serum-reduced culture [40]. In contrast, quenching of the Smad signaling pathway alone seems critical and rate-limiting for myogenic differentiation and induces myogenic differentiation under the high cell density culture condition even in the high serum-containing culture. Therefore, the two myogenic differentiation-inducing conditions, high cell density and serum reduction, may induce myogenic differentiation in different ways (Fig. 8C): suppression of the Smad signaling pathway alone is rate-limiting for myogenesis or both the Smad signaling pathway and the other signaling pathways play a role in the rate-determining step for myogenesis. It is conceivable that the Smad signaling pathway plays a role in the rate-limiting step for postnatal myogenesis in vivo. From this point of view, it is noteworthy that the Smad signaling pathway is actually down-regulated during BPV-induced muscle regeneration.

Community effect triggers terminal differentiation of postnatal myogenic cells

The present study suggests that muscle satellite cell-dependent myogenesis in postnatal mice depends on a "community effect," which means the expression of a differentiation potential when a certain cell density is exceeded [41], and also provides evidence that quenching the Smad signaling pathway in postnatal myogenic cells is required for the community effect. Skeletal muscle formation in amphibian embryos provides a paradigm of the community effect [42]. Dissociation of muscle progenitor cells reduces their differentiation, whereas the reaggregated cells differentiate [43]. In mouse embryos, muscle differentiation also depends on a community effect [44]. Previous studies on embryonic myogenesis and the present study on postnatal myogenesis both suggest that the developmental timing of a community effect is important as a critical switching mechanism between growth and differentiation of myogenic progenitor cells during embryonic and postnatal muscle growth, repair, and differentiation in mice (Fig. 8D).

Both signals from adjacent tissues and a community effect are necessary for the formation of skeletal muscle in embryos [45]. Fibroblast growth factors (FGFs) are candidates for community factors in *Xenopus* embryos [41]. During postnatal muscle growth and repair in mice and humans, muscle satellite cells and their descendant progenitor cells express and release a number of trophic factors that are candidates for community factors, including growth factors and cytokines such as BMPs, myostatin, FGFs, hepatocyte growth factor, insulin-like growth factors, interleukin-6, leukemia inhibitory factor, and tumor necrotic

factor α [21,46–52]. In embryos, BMP4 is released from the adjacent neural tube and lateral plate mesoderm, and inhibits MyoD and Myf5 gene expression [53,54]. However, the role of BMP signaling in a community effect during skeletal muscle formation in embryos remains to be determined. In contrast, the present study shows that a community effect quenches the Smad signaling pathway in postnatal myogenic cells. Thus, BMP antagonists are possible candidates for community factors. The list of BMP antagonists includes noggin, chordin, gremlin, follistatin, Cerberus, sclerostin, and their related and family proteins [55]. Careful description of the spatiotemporal expression patterns of these antagonists during myogenesis induced by high cell density may provide a hypothetical and mechanistic view of the community effect. However, the activity of the BMP–ALK–Smad axis could be also modulated by Notch signaling [56] or Src tyrosine kinase [57]. Therefore, we should observe whether a simple community factor story can explain a community effect on the terminal differentiation of postnatal myogenic cells.

The origin of ligands that stimulate the Smad signaling pathway in growing myogenic progenitor cells remains puzzling. We cannot exclude the possibility that the FBS in the medium contains an adequate amount of BMP to support the activation of Smad1/5/8, but putative BMP derived from FBS is unlikely to contribute to the activation of Smad1/5/8 in growing myogenic cells because serum reduction does not significantly affect the level of phosphorylated Smad1/5/8 except in higher density culture. In addition, the Smad signaling pathway is inactivated exclusively in the central region of a micromass, even in pmGM supplemented with 20% FBS. Therefore, BMP4 produced by myogenic progenitor cells themselves [21] is a possible candidate for ligands that stimulate their own Smad signaling pathway.

Community effect guarantees myogenic cell fusion following expression of muscle-specific genes

Skeletal muscle terminal differentiation of muscle satellite cells is composed of a highly ordered series of steps that includes activation of quiescent satellite cells, proliferation of descendent progenitor cells, expression of muscle-specific genes, and cell fusion to give rise to syncytia. Cell fusion is the last step of terminal muscle differentiation and is a multi-cellular event, whereas the other steps are uni-cellular responses. To differentiate into myotubes, a differentiating myogenic cell requires direct contact with its fusion partner cell. If a single myogenic progenitor cell is cultured without contact with other cells under the serum-reduced condition, it will undergo the myogenic differentiation process up to the expression of muscle-specific genes but be unable to form myotubes. Therefore, neighboring myogenic cells, including progenitor cells and myofibers, are required for terminal differentiation of myogenic progenitor cells. The community effect induces myogenic differentiation when a certain cell density is exceeded and guarantees myogenic cell contact relevant for syncytium formation. The community effect also provides a probable explanation of how non-synchronized and local myogenic differentiation is induced in culture when myogenic cells are distributed unevenly throughout a culture dish. In addition, high-level expression of noggin in myotubes (Hashimoto, unpublished observation) raises the possibility of differentiated cell-induced differentiation: terminally differentiated cells enhance the community effect and induce differentiation of neighboring undifferentiated myogenic cells (Fig. 8D).

Concluding remarks

We have shown a novel physiological role of the Smad signaling pathway in a switch between growth and differentiation of postnatal myogenic cells. Further studies identifying factors that quench the Smad signaling pathway will provide mechanistic insight into a community effect on postnatal myogenesis.

Supplementary materials related to this article can be found online at doi:10.1016/j.yexcr.2010.10.011.

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REFERENCES

[1] G. Cossu, U. Borello, Wnt signaling and the activation of myogenesis in mammals, EMBO J. 18 (1999) 6867–6872.
[2] G. Cossu, M. Molinaro, Cell heterogeneity in the myogenic lineage, Curr. Top. Dev. Biol. 23 (1987) 185–208.
[3] D. Elia, D. Madhala, E. Ardon, R. Reshef, O. Halevy, Sonic hedgehog promotes proliferation and differentiation of adult muscle cells: involvement of MAPK/ERK and PI3K/Akt pathways, Biochim. Biophys. Acta 1773 (2007) 1438–1446.
[4] M. Koleva, R. Kappler, M. Vogler, A. Herwig, S. Fulda, H. Hahn, Pleiotropic effects of sonic hedgehog on muscle satellite cells, Cell. Mol. Life Sci. 62 (2005) 1863–1870.
[5] G. Straface, T. Aprahamian, A. Flex, E. Gaetani, F. Biscetti, R.C. Smith, G. Pecorini, E. Pola, F. Angelini, E. Stigliano, J.J. Castellot, D.W. Losordo, R. Pola, Sonic hedgehog regulates angiogenesis and myogenesis during post-natal skeletal muscle regeneration, J. Cell Mol. Med. 13 (2009) 2424–2435.
[6] A. Otto, C. Schmidt, G. Luke, S. Allen, P. Valasek, F. Muntoni, D. Lawrence-Watt, K. Patel, Canonical Wnt signalling induces satellite-cell proliferation during adult skeletal muscle r egeneration, J. Cell Sci. 121 (2008) 2939–2950.
[7] A. Asakura, M. Komaki, M. Rudnicki, Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation, Differentiation 68 (2001) 245–253.
[8] T. Katagiri, A. Yamaguchi, M. Komaki, E. Abe, N. Takahashi, T. Ikeda, V. Rosen, J.M. Wozney, A. Fujisawa-Sehara, T. Suda, Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage, J. Cell Biol. 127 (1994) 1755–1766.
[9] M.R. Wada, M. Inagawa-Ogashiwa, S. Shimizu, S. Yasumoto, N. Hashimoto, Generation of different fates from multipotent muscle stem cells, Development 129 (2002) 2987–2995.
[10] N. Yamamoto, S. Akiyama, T. Katagiri, M. Namiki, T. Kurokawa, T. Suda, Smad1 and smad5 act downstream of intracellular signalings of BMP-2 that inhibits myogenic differentiation and induces osteoblast differentiation in C2C12 myoblasts, Biochem. Biophys. Res. Commun. 238 (1997) 574–580.
[11] R. Nishimura, Y. Kato, D. Chen, S.E. Harris, G.R. Mundy, T. Yoneda, Smad5 and DPC4 are key molecules in mediating BMP-2-induced osteoblastic differentiation of the pluripotent mesenchymal precursor cell line C2C12, J. Biol. Chem. 273 (1998) 1872–1879.
[12] M. Fujii, K. Takeda, T. Imamura, H. Aoki, T.K. Sampath, S. Enomoto, M. Kawabata, M. Kato, H. Ichijo, K. Miyazono, Roles of bone morphogenetic protein type I receptors and Smad proteins in

osteoblast and chondroblast differentiation, Mol. Biol. Cell 10 (1999) 3801-3813.
[13] H. Aoki, M. Fujii, T. Imamura, K. Yagi, K. Takehara, M. Kato, K. Miyazono, Synergistic effects of different bone morphogenetic protein type I receptors on alkaline phosphatase induction, J. Cell Sci. 114 (2001) 1483-1489.
[14] N. Hashimoto, T. Murase, S. Kondo, A. Okuda, M. Inagawa-Ogashiwa, Muscle reconstitution by muscle satellite cell descendants with stem cell-like properties, Development 131 (2004) 5481-5490.
[15] J. Nojima, K. Kanomata, Y. Takada, T. Fukuda, S. Kokabu, S. Ohte, T. Takada, T. Tsukui, T.S. Yamamoto, H. Sasanuma, K. Yoneyama, N. Ueno, Y. Okazaki, R. Kamijo, T. Yoda, T. Katagiri, Dual roles of smad proteins in the conversion from myoblasts to osteoblastic cells by bone morphogenetic proteins, J. Biol. Chem. 285 (2010) 15577-15586.
[16] N.Y. Frank, A.T. Kho, T. Schatton, G.F. Murphy, M.J. Molloy, Q. Zhan, M.F. Ramoni, M.H. Frank, I.S. Kohane, E. Gussoni, Regulation of myogenic progenitor proliferation in human fetal skeletal muscle by BMP4 and its antagonist Gremlin, J. Cell Biol. 175 (2006) 99-110.
[17] H.M. Blau, G.K. Pavlath, E.C. Hardeman, C.P. Chiu, L. Silberstein, S.G. Webster, S.C. Miller, C. Webster, Plasticity of the differentiated state, Science 230 (1985) 758-766.
[18] A. Mukai, N. Hashimoto, Localized cyclic AMP-dependent protein kinase activity is required for myogenic cell fusion, Exp. Cell Res. 314 (2008) 387-397.
[19] D. Yaffe, O. Saxel, A myogenic cell line with altered serum requirements for differentiation, Differentiation 7 (1977) 159-166.
[20] A. Mukai, T. Kurisaki, S.B. Sato, T. Kobayashi, G. Kondoh, N. Hashimoto, Dynamic clustering and dispersion of lipid rafts contribute to fusion competence of myogenic cells, Exp. Cell Res. 315 (2009) 3052-3063.
[21] N. Hashimoto, T. Kiyono, M.R. Wada, R. Umeda, Y. Goto, I. Nonaka, S. Shimizu, S. Yasumoto, M. Inagawa-Ogashiwa, Osteogenic properties of human myogenic progenitor cells, Mech. Dev. 125 (2008) 257-269.
[22] N. Hashimoto, T. Kiyono, M.R. Wada, S. Shimizu, S. Yasumoto, M. Inagawa, Immortalization of human myogenic progenitor cell clone retaining multipotentiality, Biochem. Biophys. Res. Commun. 348 (2006) 1383-1388.
[23] N. Hashimoto, M. Ogashiwa, Isolation of a differentiation-defective myoblastic cell line, INC-2, expressing muscle LIM protein under differentiation-inducing conditions, Dev. Growth Differ. 39 (1997) 363-372.
[24] N. Hashimoto, M. Ogashiwa, S. Iwashita, Role of tyrosine kinase in the regulation of myogenin expression, Eur. J. Biochem. 227 (1995) 379-387.
[25] N. Hashimoto, M. Ogashiwa, E. Okumura, T. Endo, S. Iwashita, T. Kishimoto, Phosphorylation of a proline-directed kinase motif is responsible for structural changes in myogenin, FEBS Lett. 352 (1994) 236-242.
[26] A. Fujisawa-Sehara, K. Hanaoka, M. Hayasaka, T. Hiromasa-Yagami, Y. Nabeshima, Upstream region of the myogenin gene confers transcriptional activation in muscle cell lineages during mouse embryogenesis, Biochem. Biophys. Res. Commun. 191 (1993) 351-356.
[27] O. Korchynskyi, P. ten Dijke, Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter, J. Biol. Chem. 277 (2002) 4883-4891.
[28] Y. Saito, I. Nonaka, Z. Qu, L. Balkir, J.C. van Deutekom, P.D. Robbins, R. Pruchnic, J. Huard, Initiation of satellite cell replication in bupivacaine-induced myonecrosis, Acta Neuropathol. (Berl) 88 (1994) 252-257.
[29] Y. Furukawa, N. Hashimoto, T. Yamakuni, Y. Ishida, C. Kato, M. Ogashiwa, M. Kobayashi, T. Kobayashi, I. Nonaka, H. Mizusawa, S.Y. Song, Down-regulation of an ankyrin repeat-containing protein, V-1, during skeletal muscle differentiation and its re-expression in the regenerative process of muscular dystrophy, Neuromuscul. Disord. 13 (2003) 32-41.
[30] D. Bader, T. Masaki, D.A. Fischman, Immunochemical analysis of myosin heavy chain during avian myogenesis in vivo and in vitro, J. Cell Biol. 95 (1982) 763-770.
[31] W.E. Wright, D.A. Sassoon, V.K. Lin, Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD, Cell 56 (1989) 607-617.
[32] H. Hirano, T. Watanabe, Microsequencing of proteins electrotransferred onto immobilizing matrices from polyacrylamide gel electrophoresis: application to an insoluble protein, Electrophoresis 11 (1990) 573-580.
[33] K. Miyazawa, M. Shinozaki, T. Hara, T. Furuya, K. Miyazono, Two major Smad pathways in TGF-beta superfamily signalling, Genes Cells 7 (2002) 1191-1204.
[34] P.B. Yu, D.Y. Deng, C.S. Lai, C.C. Hong, G.D. Cuny, M.L. Bouxsein, D.W. Hong, P.M. McManus, T. Katagiri, C. Sachidanandan, N. Kamiya, T. Fukuda, Y. Mishina, R.T. Peterson, K.D. Bloch, BMP type I receptor inhibition reduces heterotopic [corrected] ossification, Nat. Med. 14 (2008) 1363-1369.
[35] R. Derynck, Y.E. Zhang, Smad-dependent and Smad-independent pathways in TGF-beta family signalling, Nature 425 (2003) 577-584.
[36] K. Goto, Y. Kamiya, T. Imamura, K. Miyazono, K. Miyazawa, Selective inhibitory effects of Smad6 on bone morphogenetic protein type I receptors, J. Biol. Chem. 282 (2007) 20603-20611.
[37] R. Benezra, R.L. Davis, D. Lockshon, D.L. Turner, H. Weintraub, The protein Id: a negative regulator of helix-loop-helix DNA binding proteins, Cell 61 (1990) 49-59.
[38] Y. Jin, N. Murakami, Y. Saito, Y. Goto, K. Koishi, I. Nonaka, Expression of MyoD and myogenin in dystrophic mice, mdx and dy, during regeneration, Acta Neuropathol. 99 (2000) 619-627.
[39] H.K. Kim, Y.S. Lee, U. Sivaprasad, A. Malhotra, A. Dutta, Muscle-specific microRNA miR-206 promotes muscle differentiation, J. Cell Biol. 174 (2006) 677-687.
[40] C.J. Liu, B. Ding, H. Wang, P. Lengyel, The MyoD-inducible p204 protein overcomes the inhibition of myoblast differentiation by Id proteins, Mol. Cell. Biol. 22 (2002) 2893-2905.
[41] M. Buckingham, How the community effect orchestrates muscle differentiation, Bioessays 25 (2003) 13-16.
[42] J.B. Gurdon, P. Lemaire, K. Kato, Community effects and related phenomena in development, Cell 75 (1993) 831-834.
[43] J.B. Gurdon, E. Tiller, J. Roberts, K. Kato, A community effect in muscle development, Curr. Biol. 3 (1993) 1-11.
[44] G. Cossu, R. Kelly, S. Di Donna, E. Vivarelli, M. Buckingham, Myoblast differentiation during mammalian somitogenesis is dependent upon a community effect, Proc. Natl Acad. Sci. USA 92 (1995) 2254-2258.
[45] G. Cossu, S. Tajbakhsh, M. Buckingham, How is myogenesis initiated in the embryo? Trends Genet. 12 (1996) 218-223.
[46] S.B.P. Charge, M.A. Rudnicki, Cellular and molecular regulation of muscle regeneration, Physiol. Rev. 84 (2004) 209-238.
[47] E. Sterrenburg, C.G. van der Wees, S.J. White, R. Turk, R.X. de Menezes, G.J. van Ommen, J.T. den Dunnen, P.A. t Hoen, Gene expression profiling highlights defective myogenesis in DMD patients and a possible role for bone morphogenetic protein 4, Neurobiol. Dis. 23 (2006) 228-236.
[48] J.R. Florini, D.Z. Ewton, K.A. Magri, Hormones, growth factors, and myogenic differentiation, Annu. Rev. Physiol. 53 (1991) 201-216.
[49] L. Sun, K. Ma, H. Wang, F. Xiao, Y. Gao, W. Zhang, K. Wang, X. Gao, N. Ip, Z. Wu, JAK1-STAT1-STAT3, a key pathway promoting proliferation and preventing premature differentiation of myoblasts, J. Cell Biol. 179 (2007) 129-138.
[50] J.W. Moore, C. Dionne, M. Jaye, J.L. Swain, The mRNAs encoding acidic FGF, basic FGF and FGF receptor are coordinately downregulated during myogenic differentiation, Development 111 (1991) 741-748.

[51] P. Baron, D. Galimberti, L. Meda, E. Scarpini, G. Conti, F. Cogiamanian, G. Scarlato, Production of IL-6 by human myoblasts stimulated with Abeta: relevance in the pathogenesis of IBM, Neurology 57 (2001) 1561–1565.
[52] M. Thomas, B. Langley, C. Berry, M. Sharma, S. Kirk, J. Bass, R. Kambadur, Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation, J. Biol. Chem. 275 (2000) 40235–40243.
[53] M. Buckingham, L. Bajard, T. Chang, P. Daubas, J. Hadchouel, S. Meilhac, D. Montarras, D. Rocancourt, F. Relaix, The formation of skeletal muscle: from somite to limb, J. Anat. 202 (2003) 59–68.
[54] X. Shi, D.J. Garry, Muscle stem cells in development, regeneration, and disease, Genes Dev. 20 (2006) 1692–1708.
[55] W. Balemans, W. Van Hul, Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators, Dev. Biol. 250 (2002) 231–250.
[56] T. Minamizato, K. Sakamoto, T. Liu, H. Kokubo, K. Katsube, B. Perbal, S. Nakamura, A. Yamaguchi, CCN3/NOV inhibits BMP-2-induced osteoblast differentiation by interacting with BMP and Notch signaling pathways, Biochem. Biophys. Res. Commun. 354 (2007) 567–573.
[57] O. Gautschi, C.G. Tepper, P.R. Purnell, Y. Izumiya, C.P. Evans, T.P. Green, P.Y. Desprez, P.N. Lara, D.R. Gandara, P.C. Mack, H.J. Kung, Regulation of Id1 expression by SRC: implications for targeting of the bone morphogenetic protein pathway in cancer, Cancer Res. 68 (2008) 2250–2258.

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ORIGINAL ARTICLE

Plasma sex hormone levels and mortality in disabled older men and women

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Aim: To investigate the relationship between circulating sex hormone levels and subsequent mortality in disabled elderly.

Methods: This prospective observational study was comprised of 214 elderly subjects aged 70–96 years (117 men and 97 women; mean ± standard deviation age, 83 ± 7 years), receiving services at long-term care facilities in Nagano, Japan. All-cause mortality by baseline plasma sex hormone levels was measured.

Results: After excluding deaths during the first 6 months, 27 deaths in men and 28 deaths in women occurred during a mean follow up of 32 months and 45 months (up to 52 months), respectively. Mortality rates differed significantly between high and low testosterone tertiles in men, but did not differ significantly between middle and low tertiles. Compared with subjects in the middle and high tertiles, men with testosterone levels in the low tertile (<300 ng/dL) were more likely to die, independent of age, nutritional status, functional status and chronic disease (hazard ratio [HR] = 3.27, 95% confidence interval [CI] = 1.24–12.91). In contrast, the low dehydroepiandrosterone sulfate (DHEA-S) tertile was associated with higher mortality risk in women (multivariate adjusted HR = 4.42, 95% CI = 1.51–12.90). Exclusion of deaths during the first year and cancer deaths had minimal effects on these results. DHEA-S level in men and testosterone and estradiol levels in women were not related to mortality.

Conclusion: Low testosterone in men and low DHEA-S in women receiving care at facilities are associated with increased mortality risk, independent of other risk factors and pre-existing health conditions. **Geriatr Gerontol Int 2010; 10: ••–••.**

Keywords: dehydroepiandrosterone, disabled elderly, mortality risk, testosterone.

Introduction

Japan has the longest life expectancy at birth in the world for both men and women, although women live 8 years longer than men on average.[1,2] One explanation for this phenomenon is that estradiol production during the premenopausal years partially protects women from cardiovascular disease (CVD). In contrast, there has been a suspicion that testosterone itself is harmful; however, recent studies support the hypothesis that testosterone may be beneficial to survival in aging men.[3–8]

It is well established that endogenous androgens decline with advancing age in men.[9] Because testosterone has important physiological effects on muscle, bone, brain, erythropoietin and the vascular system, decreased testosterone levels could contribute to age-associated symptoms and diseases in older men, such as decreased muscle mass and strength,[10] impaired physical performance,[11,12] osteoporosis[13] and fractures,[12,14]

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depressed mood,[15] cognitive impairment,[16,17] anemia[18,19]and frailty.[20] In our previous study in which older persons receiving day-care services or admitted to a facility were investigated, higher plasma testosterone levels were associated with better activities of daily living (ADL), cognitive function and vitality in men.[21] On the other hand, several epidemiological studies have demonstrated that a decline in testosterone level was associated with mortality risk in community-dwelling middle-aged or older men.[3–8] In cause-specific analyses, some studies have shown that a low testosterone level was associated with an increased risk of death due to CVD.[4,5] However, the above-mentioned studies were performed in community samples of Caucasian men, and this issue remains to be clarified in frail or disabled older men.

The majority of dehydroepiandrosterone (DHEA), an endogenous steroid precursor to testosterone and estrogen, exists as the sulfated form (DHEA-S) in the circulation, and DHEA and DHEA-S are the most abundant adrenal sex steroid hormones, with concentrations reported to be more than 100-fold higher than those of testosterone and estradiol,[22] suggesting an important physiological role of DHEA(-S). Their circulating levels also peak in young adults and decline with age in both men and women. Although the role of androgens in older women's health is not fully understood, postmenopausal women with intact ovaries continue to produce androgens, DHEA and testosterone, while their production of estradiol is minimal.[23] In our previous study,[21] in older women, higher DHEA and DHEA-S levels were related to better ADL, while estradiol and testosterone levels showed no relations. Other reports have shown a correlation between DHEA level and cognitive function,[24] depression,[25] osteoporosis[26] and frailty in older women.[27] Several studies that examined the association between DHEA-S and mortality in women have shown mixed results,[28–32] and mostly found no relation; however, both low and high levels of DHEA-S at baseline[28] and some trajectory patterns such as a steep decline or extreme variability[32] have been reported to correlate with increased mortality.

These lines of evidence suggest that endogenous androgens, including testosterone and DHEA(-S), may play a role in physical and mental function as well as longevity in older individuals. We hypothesized that low plasma androgen levels could be a mortality risk factor even in elderly with disability who are receiving facility services.

Methods

Study population

In this longitudinal observational study, 218 consecutive persons aged 70 years or older (121 men aged 70–96 years and 97 women aged 70–95 years; mean ± standard deviation [SD] age, 83 ± 6 and 83 ± 5 years, respectively) who attended health service facilities for the elderly (facilities that provide nursing care and rehabilitation services to elderly people with disability, *Mahoroba-no-Sato*) located in Nagano Prefecture, Japan were enrolled. The participants were in a chronic stable condition and receiving services under Long-term Care Insurance, which is provided by the Japanese Government, either under admission or as day care. The principal exclusion criteria were malnutrition (serum albumin <3.5 mg/dL or body mass index [BMI] <16 kg/m^2), extremely low ADL status (Barthel Index[33] <50), malignancy, acute inflammation (fever, white blood cell count >10 000/μL, or other signs of infection within 4 weeks before enrollment), severe anemia (blood hemoglobin <10.0 g/dL) and overt endocrine disease because these conditions may affect both plasma sex hormone levels and mortality. Deaths that occurred during the first 6 months of follow up (four men and no women) were also excluded to minimize the influence of comorbidity on both sex hormone levels and mortality; therefore, the remaining 214 persons were analyzed in this study. The institutional review board of *Mahoroba-no-Sato* approved the study protocol, and all participants and/or their family members gave written informed consent.

Hormone measurements

Blood samples were obtained from the participants in the morning after an overnight fast, and plasma hormone levels in addition to blood cell counts and blood chemical parameters were determined by a commercial laboratory (Health Sciences Research Institute, Yokohama, Japan). Testosterone and estradiol were assayed using chemiluminescence immunoassays with minimum detection limits of 7 ng/dL (0.2 nmol/L) and 4 pg/mL (14.7 pmol/L), respectively. DHEA-S was assayed using a sensitive radioimmunoassay with a minimum detection limit of 2.0 μg/dL (0.05 μmol/L). The intra-assay coefficients of variation for these measurements were less than 5%.

Functional and anthropometric measurements

Trained nurses and physical therapists visited the participants at the health facilities and performed comprehensive geriatric assessments. Basic ADL was assessed by Barthel Index,[33] cognitive function by Hasegawa Dementia Scale – Revised (HDS-R, 30-point scale),[34] mood by the Geriatric Depression Scale (GDS, 15 items),[35] and ADL-related vitality by Vitality Index (10-point scale).[36] BMI was calculated

as weight in kilograms divided by the square of height in meters.

Comorbidity

Diseases were ascertained by experienced physicians according to pre-established criteria that combine information from self-reported physician diagnoses, medical records, current medication, clinical examinations and blood tests. Diseases included in the current analysis were hypertension, heart disease (including any of angina pectoris, myocardial infarction, congestive heart failure and arrhythmia), stroke, diabetes mellitus, osteoarthropathy (arthritis, rheumatism, osteoporosis and history of fractures), lung disease (including bronchial asthma and chronic obstructive pulmonary disease) and other chronic diseases (chronic kidney disease, gastrointestinal disease, Parkinson's disease and psychological disorders). We also obtained data on anti-androgenic treatment and intake of glucocorticoids, opiates and hormone supplements that could affect plasma hormone levels, but no subject was taking any of these.

Follow up

The subjects were followed up in 2002–2009, for a period of up to 52 months (mean ± SD, 32 ± 13 [34] months in men and 45 ± 11 [49] months in women). Time and causes of death of deceased persons were ascertained using medical records and death certificates. All deaths were registered with International Classification of Diseases, 10th version (ICD-10) codes,[37] based on the information from death certificates. We categorized deaths into the following four specific causes: (i) diseases of the circulatory system (I00–I99) including heart disease and cerebrovascular disease; (ii) diseases of the respiratory system (J00-J99); (iii) neoplasms (C00-D48); and (iv) other causes. Subjects who were alive were confirmed by checking appointment records of the facilities. Survival of 16 subjects whose records were not available was ascertained by the phone interview of each subject. Causes of death were determined for all the subjects without any missing cases.

Statistical analysis

Differences between testosterone tertiles in men and between DHEA-S tertiles in women were analyzed using ANOVA for continuous variables and χ^2-test for categorical variables. Survival was analyzed using Kaplan–Meier plots and log–rank tests. Hazard ratios (HR) for mortality were analyzed using Cox proportional hazards regression. Significance tests were two-sided, with an α-level of 0.05. Data were analyzed using SPSS statistical software.

Results

Characteristics of study subjects

Over the follow-up period, 27 men and 28 women died, yielding a mortality rate of 86.5/1000 person-years at risk in men; and 69.9/1000 person-years at risk in women. Of those, 13 deaths were due to diseases of the circulatory system (eight to ischemic and other heart disease and five to cerebrovascular disease), 10 to diseases of the respiratory system and four to cancer in men; while 14 deaths were due to diseases of the circulatory system (nine to ischemic and other forms of heart disease and four to cerebrovascular disease), eight to diseases of the respiratory system, five to cancer and two to other causes in women. Men who died were significantly older, had lower serum albumin and cholesterol, lower ADL and cognitive status, higher prevalence of heart disease, and lower testosterone level than survivors; whereas in women, subjects who died were older, had lower hemoglobin, higher prevalence of heart disease and lower plasma DHEA-S level than survivors (data not shown).

Table 1 shows the baseline characteristics of the male subjects by tertile of plasma testosterone. A significant difference was observed in serum albumin and hemoglobin levels, ADL and cognitive status among tertiles of testosterone in men. Table 2 shows the baseline characteristics of the female subjects by tertile of plasma DHEA-S. A significant difference was found in age and ADL status among DHEA-S tertiles in women, while other variables did not differ between the tertile groups.

Mortality and plasma sex hormone levels in men

As shown in Figure 1(a), Kaplan–Meier survival analysis by tertile of plasma testosterone level revealed that testosterone level was associated with mortality in men. After adjusting for age, Cox proportional hazards models showed that there was an inverse relation between testosterone level and mortality. Mortality rate differed significantly between the high and low testosterone tertiles, but not significantly between the middle and low tertiles: tertile 3 (high), reference; tertile 2 (middle), HR = 2.51 (95% confidence interval [CI] = 0.66–9.50); and tertile 1 (low), HR = 6.63 (95% CI = 1.92–23.21). Accordingly, we investigated the increased mortality in tertile 1 versus tertiles 2–3 (Table 3). Compared with subjects within tertiles 2–3,

Table 1 Association between potential confounding variables and testosterone tertiles in men

Characteristic	Testosterone tertiles T1 <10.4 nmol/L (<300 ng/dL), *n* = 39	T2 10.4–16.3 nmol/L (300–470 ng/dL), *n* = 40	T3 >16.3 nmol/L (>470 ng/dL), *n* = 38	*P*-value
Age, years	83 ± 7	83 ± 6	81 ± 6	0.11
Nutritional parameters				
Body mass index, kg/m^2	21.3 ± 3.4	22.8 ± 3.8	21.7 ± 3.0	0.21
Hemoglobin, g/dL	12.7 ± 1.9	13.8 ± 1.3	14.0 ± 1.7	<0.01
Albumin, g/dL	4.0 ± 0.3	4.1 ± 0.2	4.2 ± 0.3	<0.01
Total cholesterol, mg/dL	173 ± 38	195 ± 36	176 ± 28	0.05
Prevalent diseases, *n* (%)				
Hypertension	17 (44)	16 (40)	12 (32)	0.53
Heart disease	10 (26)	5 (13)	7 (18)	0.32
Stroke	12 (31)	15 (38)	8 (21)	0.34
Diabetes mellitus	8 (21)	5 (13)	8 (21)	0.31
Osteoarthropathy	8 (21)	9 (23)	7 (18)	0.94
Lung disease	2 (5)	3 (8)	3 (8)	0.52
Other chronic diseases	17 (44)	19 (48)	18 (47)	0.95
Functional parameters				
Barthel Index	79 ± 12	82 ± 11	87 ± 13	0.04
HDS-R	18 ± 7	19 ± 6	22 ± 5	0.02
Vitality Index	9.2 ± 1.1	9.3 ± 0.9	9.5 ± 0.9	0.46
GDS	5.0 ± 3.1	5.6 ± 3.7	5.6 ± 2.9	0.66
Sex hormone levels				
Testosterone, nmol/L	7.6 ± 2.5	13.3 ± 1.6	20.9 ± 3.9	<0.01
(ng/dL)	(219 ± 73)	(382 ± 43)	(602 ± 112)	
DHEA-S, μmol/L	1.7 ± 1.1	1.8 ± 1.6	1.7 ± 1.2	0.94
(μg/dL)	(64 ± 42)	(69 ± 57)	(63 ± 45)	

Values are shown as mean (standard deviation). Differences between the groups were analyzed using ANOVA for continuous variables and χ^2-test for categorical variables. DHEA-S, dehydroepiandrosterone sulfate; GDS, Geriatric Depression Scale; HDS-R, Hasegawa Dementia Scale – Revised.

a testosterone level within tertile 1 was associated with approximately fourfold higher mortality risk. Adjustment for age, nutritional parameters (BMI, albumin, hemoglobin, total cholesterol) and functional parameters (Barthel Index, HDS-R, Vitality Index, GDS), and prevalent diseases showed no major influence on the result. In order to examine how follow-up time and cancer impacted on the results, assuming that the subjects may have had subclinical cancer or a fatal illness at baseline, we performed further analyses excluding deaths that occurred in the first 12 months ($n = 9$) and deaths from cancer ($n = 4$). However, the significant associations remained after these exclusions (Table 3). On the other hand, DHEA-S level was not associated with mortality when DHEA-S was entered as tertiles (data not shown).

Although the statistical power was not strong enough, we studied the risk for cause-specific mortality by tertiles of testosterone level in men. Neither deaths from diseases of the circulatory system nor those from non-circulatory causes showed a significant association with testosterone tertiles (tertile 1 vs tertile 2–3, HR = 3.18, 95% CI = 1.87–11.6, $P = 0.17$; HR = 3.46, 95% CI = 0.29–7.29, $P = 0.64$, respectively).

Mortality and plasma sex hormone levels in women

As shown in Figure 1(b), a low DHEA-S level was associated with higher mortality by Kaplan–Meier survival analysis. Age-adjusted Cox proportional hazards models revealed that the association was not significant when each tertile of DHEA-S was entered as a continuous variable; however, a significant association was observed when tertile 1 was compared with tertiles 2–3 (Table 3). The association remained significant after excluding deaths that occurred in the first 12 months ($n = 2$) and deaths from cancer ($n = 5$). Moreover, further adjustment had no major influence on the result. In women, testosterone and estradiol levels were not associated with mortality when they were entered as tertiles (data not shown).

In cause-specific mortality analysis, compared with tertiles 2–3, the low tertile of DHEA-S level was associated with higher risk of death from diseases of the

Table 2 Association between potential confounding variables and DHEA-S tertiles in women

Characteristic	DHEA-S tertiles T1 <1.17 μmol/L (<43 μg/dL), *n* = 33	T2 1.17–1.49 μmol/L (43–55 μg/dL), *n* = 32	T3 >1.49 μnmol/L (>55 μg/dL), *n* = 32	*P*-value
Age, years	83 ± 6	82 ± 6	80 ± 6	0.08
Nutritional parameters				
Body mass index, kg/m^2	22.3 ± 2.7	22.5 ± 3.2	23.7 ± 2.7	0.31
Hemoglobin, g/dL	12.6 ± 1.4	12.6 ± 1.2	13.1 ± 1.1	0.16
Albumin, g/dL	4.1 ± 0.3	4.2 ± 0.3	4.3 ± 0.2	0.18
Total cholesterol, mg/dL	205 ± 30	204 ± 35	205 ± 35	0.99
Prevalent diseases, *n* (%)				
Hypertension	10 (30)	14 (44)	15 (47)	0.47
Heart disease	4 (12)	7 (22)	8 (25)	0.46
Stroke	5 (15)	4 (13)	6 (19)	0.79
Diabetes mellitus	5 (15)	4 (13)	5 (16)	0.90
Osteoarthropathy	8 (24)	11 (34)	13 (40)	0.47
Lung disease	3 (9)	2 (6)	2 (6)	0.56
Other chronic diseases	17 (52)	19 (59)	18 (56)	0.90
Functional parameters				
Barthel Index	90 ± 7	93 ± 8	95 ± 8	0.04
HDS-R	23 ± 6	22 ± 7	25 ± 5	0.39
Vitality Index	9.2 ± 1.4	9.1 ± 2.2	8.8 ± 2.9	0.35
GDS	6.8 ± 2.6	5.9 ± 3.4	6.9 ± 3.3	0.16
Sex hormone levels				
DHEA-S, μmol/L	0.8 ± 0.2	1.3 ± 0.1	2.0 ± 0.3	<0.01
(μg/dL)	30 ± 7	49 ± 4	73 ± 12	
Testosterone, nmol/L	1.2 ± 0.6	1.2 ± 0.6	1.3 ± 0.5	0.81
(ng/dL)	35 ± 17	36 ± 17	37 ± 13	
Estradiol, pmol/L	56 ± 32	57 ± 37	67 ± 46	0.41
(pg/mL)	15.3 ± 8.6	15.5 ± 10.2	18.3 ± 12.5	

Values are shown as mean (standard deviation). Differences between the groups were analyzed using ANOVA for continuous variables and χ^2-test for categorical variables. DHEA-S, dehydroepiandrosterone sulfate; GDS, Geriatric Depression Scale; HDS-R, Hasegawa Dementia Scale – Revised.

circulatory system (HR = 13.1, 95% CI = 2.39–72.3, $P < 0.01$), while there was no association with deaths from non-circulatory causes (HR = 0.93, 95% CI = 0.86–1.02, $P = 0.14$).

Discussion

In this small prospective study of Japanese elderly who were receiving care in facilities, a low testosterone level was associated with mortality in men independent of multiple risk factors and pre-existing health conditions. In addition, a low DHEA-S level in older women was related to increased mortality. In contrast, DHEA-S level in men and testosterone and estradiol levels in women were not related to mortality.

Recent prospective cohort studies in Western countries have yielded inconsistent findings about the use of a low total testosterone level as a predictor of all-cause and cardiovascular mortality in middle-aged to older men.[4,5,38,39] In the two studies that found no significant prediction of mortality,[38,39] the populations were younger (mean or median ages were in the early 50s), testosterone levels were higher and mortality rates were lower (11.6 and 15.4/1000 person-years, respectively) compared to those in studies that found positive results. In the present study, although the sample size was small, the subjects were frail and older than those in any previously reported studies, with a relatively small age range and higher mortality rate. Therefore, the relation between testosterone level and mortality might have been easier to detect in our study than in other studies with healthy middle-aged and older men.

There could be several mechanisms by which endogenous testosterone affects mortality in men. Although the number of subjects was too small to perform cause-specific analysis in the present study, other studies have reported that a low testosterone level predicted increased risk of death due to CVD.[4,5] Further, in addition to the relation to muscle strength, physical performance and ADL,[10–12,21] some but not all reports have

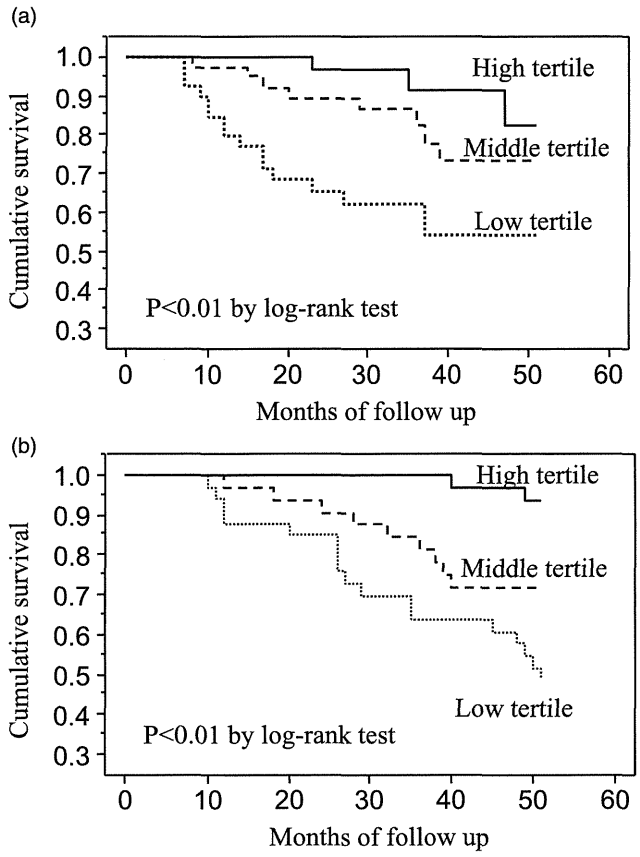

Figure 1 (a) Survival curves by tertile group of plasma testosterone level in men. (b) Survival curves by tertile group of plasma dehydroepiandrosterone sulfate level in women.

demonstrated an association between low testosterone level in older men and risk of a fall or fracture and frailty.[12–14,20] It is noteworthy that in the 10 men who died of respiratory infection, four had a history of a fall and fracture, which resulted in worse disability. Accordingly, a low testosterone level may contribute to frailty, which influences men's susceptibility to illness and falls and the capability to recover from disease or fractures, and thereby affects mortality.

Other than aging, systemic illness can result in decreased testosterone levels; therefore, low testosterone levels in older men could be attributable to acute and chronic diseases,[40] and the possible reverse causality should be considered. To evaluate this possibility, we excluded the first 12 months of observation and still found that in 12–52 months of observation, men in the low testosterone tertile had a greater risk of mortality from all causes than those in higher tertiles. We carefully excluded subjects with critical diseases and conditions at baseline, although our subjects were old with multiple chronic diseases, and it is difficult to exclude the possibility that men with subclinical critical conditions might have been included. Moreover, at baseline, there was a significant difference in functional status (ADL and cognition) and nutritional parameters (serum albumin and hemoglobin levels) between testosterone tertiles, as reported previously;[21] thus, our results need to be confirmed in a cohort with no difference in these factors between testosterone groups to exclude the influence of these biases on mortality. Also, it needs to be explored whether low testosterone in older men plays a pathogenic role, such as affecting the immune system, developing physical frailty and depression, or simply serves as a marker for biological vulnerability and poor prognosis. Long-term studies also need to test whether testosterone treatment should yield clinically significant improvements in mortality in appropriately selected older men, with consistent symptoms and signs and unequivocally low serum testosterone levels.

Low DHEA-S has been associated with increased all-cause and cardiovascular mortality in older men;[26,27,41] however, no association was found in the present study. Because DHEA(-S) is an inactive prohormone and we and others have found an association between testosterone and mortality,[3–8] it is suggested that testosterone could be a stronger predictor of mortality in older men.

On the other hand, a low DHEA-S level in older women was associated with a poor prognosis after adjusting for multiple factors related to mortality. Other previous reports showed an inconsistent relationship between DHEA-S level and mortality in older women,[29–31] possibly due to differences in the cohorts including age, DHEA-S level, heterogeneity of health status and mortality rate, and the method of statistical analysis used to demonstrate the relationship, regression models with linear/non-linear assumption.

Previous studies support a potential physiological role of DHEA-S, which could contribute to reduced mortality, an anti-inflammatory action and immune regulatory activity.[42] However, there are still many unanswered questions regarding DHEA's role in aging, and there is insufficient evidence to support DHEA replacement for increasing longevity in older women. It also needs to be explored whether the DHEA-S level contributes to mortality or is merely a biomarker of the underlying health condition of older women.

Our study has some limitations. First, the sample size was too small to reach a clear conclusion with strong statistical power, thus limiting the precision of the estimates, which is reflected in the broad range of HR for mortality. Second, the results are based on single measurements of sex hormones, which do not allow assessment of changes in levels over time; therefore, they may overestimate or underestimate the association between hormone levels and mortality. Third, we did not measure estradiol levels in men, although it would have been helpful to see whether the effects of testosterone on mortality are mediated by testosterone itself or by aromatization to estradiol in older men. Finally, active forms of testosterone such as bioavailable and

Table 3 Hazard ratios for low tertile 1 vs tertiles 2–3 of plasma sex hormone levels for all-cause mortality in men and women

	Unadjusted	Model 1	Model 2
Men (n = 117)			
HR of low testosterone for mortality	3.83 (1.74–8.40)**	3.71 (1.54–8.04)**	3.27 (1.24–12.91)*
Excluding first-year deaths (n = 108)	3.81 (1.53–6.93)**	3.49 (1.14–7.39)**	3.08 (1.11–13.62)*
Excluding deaths from cancer (n = 113)	4.18 (1.77–9.86)**	4.03 (1.70–9.58)**	5.02 (1.51–15.41)*
Women (N = 97)			
HR of low DHEA-S for mortality	3.77 (1.77–8.07)**	3.86 (1.79–8.32)**	4.42 (1.51–12.90)*
Excluding first-year deaths (n = 95)	3.38 (1.55–7.37)**	3.43 (1.56–9.54)**	3.58 (1.12–11.46)*
Excluding deaths from cancer (n = 92)	3.82 (1.69–8.60)**	3.55 (1.54–8.19)**	3.92 (1.28–11.98)*

*P < 0.05; **P < 0.01 vs reference group (tertile 2–3). Values are expressed as HR (95% CI). Model 1, adjusted for age; Model 2, adjusted for age, nutritional parameters, functional parameters and prevalent disease. DHEA-S, dehydroepiandrosterone sulfate; HR, hazards ratio.

calculated free testosterone were not measured, because a direct assay of bioavailable testosterone or an assay of sex hormone binding globulin, which is necessary for free testosterone calculation, is not available in Japan. However, because most of the above-mentioned previous reports have shown an association of total testosterone with mortality, the fundamental findings might not have differed if active forms of testosterone had been analyzed.

In conclusion, a low testosterone level in men and a low DHEA-S level in women are associated with increased mortality risk, independent of multiple risk factors and several pre-existing health conditions in disabled elderly. To our knowledge, the present study is the first that showed testosterone as a predictor of mortality in Asian men. Also, this is the first study that investigated frail or disabled older persons receiving care at facilities. Our results imply the clinical importance of measuring plasma androgen levels even in disabled elderly to estimate their prognosis.

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References

1 UN. World population prospects: the 2008 revision. [Cited 1 Mar 2010.] Available from URL: http://www.un.org/esa/population/
2 Statistics and Information Department Minister's Secretariat Ministry of Health, Labour and Welfare Japanese Government. Abridged life tables for Japan. 2008 Ministry of Health, Labour and Welfare. [Cited 1 Mar 2010.] Available from URL: http://www.mhlw.go.jp
3 Shores MM, Matsumoto AM, Sloan KL, Kivlahan DR. Low serum testosterone and mortality in male veterans. *Arch Intern Med* 2006; **166**: 1660–1665.
4 Khaw KT, Dowsett M, Folkerd E *et al.* Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) Prospective Population Study. *Circulation* 2007; **116**: 2694–2701.
5 Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. *J Clin Endocrinol Metab* 2008; **93**: 68–75.
6 Shores MM, Moceri VM, Gruenewald DA, Brodkin KI, Matsumoto AM, Kivlahan DR. Low testosterone is associated with decreased function and increased mortality risk: a preliminary study of men in geriatric rehabilitation unit. *J Am Geriatr Soc* 2004; **52**: 2077–2081.
7 Tivesten A, Vandenput L, Labrie F *et al.* Low serum testosterone and estradiol predict mortality in elderly men. *J Clin Endocrinol Metab* 2009; **94**: 2482–2488.
8 Vikan T, Schirmer H, Njølstad I, Svartberg J. Endogenous sex hormones and the prospective association with cardiovascular disease and mortality in men: the Tromsø Study. *Eur J Endocrinol* 2009; **161**: 435–442.
9 Muller M, den Tonkelaar I, Thijssen JH, Grobbee DE, van der Schouw YT. Endogenous sex hormones in men aged 40–80 years. *Eur J Endocrinol* 2003; **149**: 583–589.
10 Schaap LA, Pluijm SM, Smit JH *et al.* The association of sex hormone levels with poor mobility, low muscle strength and incidence of falls among older men and women. *J Clin Endocrinol* 2005; **63**: 152–160.
11 O'Donnell AB, Travison TG, Harris SS, Tenover JL, McKinlay JB. Testosterone, dehydroepiandrosterone, and physical performance in older men: results from the Massachussetts Male Aging Study. *J Clin Endocrinol Metab* 2006; **91**: 425–431.
12 Orwoll E, Lambert LC, Marshall LM *et al.* Endogenous testosterone levels, physical performance, and fall risk in older men. *Arch Intern Med* 2006; **166**: 2124–2131.
13 Fink HA, Ewing SK, Ensrud KE *et al.* Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. *J Clin Endocrinol Metab* 2006; **91**: 3908–3915.

14 Meier C, Nguyen TV, Handelsman DJ *et al.* Endogenous sex hormones and incident fracture risk in older men: the Dubbo Osteoporosis Epidemiology Study. *Arch Intern Med* 2008; **168**: 47–54.
15 Almeida OP, Yeap BB, Hankey GJ, Jamrozik K, Flicker L. Low free testosterone concentration as a potentially treatable cause of depressive symptoms in older men. *Arch Gen Psychiatry* 2008; **65**: 283–289.
16 Yaffe K, Lui LY, Zmuda J, Cauley J. Sex hormones and cognitive function in older men. *J Am Geriatr Soc* 2002; **50**: 707–712.
17 Moffat SD, Zonderman AB, Metter EJ, Blackman MR, Harman SM, Resnick SM. Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. *J Clin Endocrinol Metab* 2002; **87**: 5001–5007.
18 Yeap BB, Beilin J, Shi Z *et al.* Serum testosterone levels correlate with haemoglobin in middle-aged and older men. *Intern Med J* 2009; **39**: 532–538.
19 Ferrucci L, Maggio M, Bandinelli S *et al.* Low testosterone levels and the risk of anemia in older men and women. *Arch Intern Med* 2006; **166**: 1380–1388.
20 Cawthon PM, Ensrud KE, Laughlin GA *et al.* Osteoporotic Fractures in Men (MrOS) Research Group. Sex hormones and frailty in older men: the osteoporotic fractures in men (MrOS) study. *J Clin Endocrinol Metab* 2009; **94**: 3806–3815.
21 Fukai S, Akishita M, Yamada S *et al.* Association of plasma sex hormone levels with functional decline in elderly men and women. *Geriatr Gerontol Int* 2009; **9**: 282–289.
22 Labrie F. Adrenal androgens and intracrinology. *Semin Reprod Med* 2004; **22**: 299–309.
23 Arlt W. Androgen therapy in women. *Eur J Endocrinol* 2006; **154**: 1–11.
24 Davis SR, Shah SM, McKenzie DP, Kulkarni J, Davison SL, Bell RJ. Dehydroepiandrosterone sulfate levels are associated with more favorable cognitive function in women. *J Clin Endocrinol Metab* 2008; **93**: 801–808.
25 Barrett-Connor E, von Mühlen D, Laughlin GA, Kripke A. Endogenous levels of dehydroepiandrosterone sulfate, but not other sex hormones, are associated with depressed mood in older women: the Rancho Bernardo Study. *J Am Geriatr Soc* 1999; **47**: 685–691.
26 Greendale GA, Edelstein S, Barrett-Connor E. Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study. *J Bone Miner Res* 1997; **12**: 1833–1843.
27 Voznesensky M, Walsh S, Dauser D, Brindisi J, Kenny AM. The association between dehydroepiandosterone and frailty in older men and women. *Age Ageing* 2009; **38**: 401–406.
28 Cappola AR, Xue QL, Walston JD *et al.* DHEAS levels and mortality in disabled older women: the Women's Health and Aging Study I. *J Gerontol A Biol Sci Med Sci* 2006; **61**: 957–962.
29 Glei DA, Goldman N. Dehydroepiandrosterone sulfate (DHEAS) and risk for mortality among older Taiwanese. *Ann Epidemiol* 2006; **16**: 510–515.
30 Mazat L, Lafont S, Berr C *et al.* Prospective measurements of dehydroepiandrosterone sulfate in a cohort of elderly subjects: relationship to gender, subjective health, smoking habits, and 10-year mortality. *Proc Natl Acad Sci U S A* 2001; **98**: 8145–8150.
31 Trivedi DP, Khaw KT. Dehydroepiandrosterone sulfate and mortality in elderly men and women. *J Clin Endocrinol Metab* 2001; **86**: 4171–4177.
32 Cappola AR, O'Meara ES, Guo W, Bartz TM, Fried LP, Newman AB. Trajectories of dehydroepiandrosterone sulfate predict mortality in older adults: the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci* 2009; **64**: 1268–1274.
33 Mahoney FI, Barthel DW. Functional evaluation: Barthel Index. *Md State Med J* 1965; **14**: 61–65.
34 Kato S, Shimogaki M, Onodera H *et al.* Revised Hasegawa Dementia Scale (HDS-R). *Jpn J Geriatr Psychiatr* 1991; **2**: 1339–1347.
35 Yesavage JA. Geriatric Depression Scale. *Psychopharmacol Bull* 1988; **24**: 709–711.
36 Toba K, Nakai R, Akishita M *et al.* Vitality Index as a useful tool to assess elderly with dementia. *Geriatr Gerontol Int* 2002; **2**: 23–29.
37 World Health Organization. *International Statistical Classification of Diseases*, 10th Revision (ICD-10). Geneva, Switzerland: World Health Organization, Version for 2007.
38 Smith GD, Ben-Shlomo Y, Beswick A, Yarnell J, Lightman S, Elwood P. Cortisol, testosterone, and coronary heart disease: prospective evidence from the Caerphilly study. *Circulation* 2005; **112**: 332–340.
39 Araujo AB, Kupelian V, Page ST, Handelsman DJ, Bremner WJ, McKinlay JB. Sex steroids and all-cause and cause-specific mortality in men. *Arch Intern Med* 2007; **167**: 1252–1260.
40 Matsumoto AM. Andropause: clinical implications of the decline in serum testosterone levels with aging in men. *J Gerontol A Biol Sci Med Sci* 2002; **57A**: M76–M99.
41 Barrett-Connor E, Khaw KT, Yen SS. A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med* 1986; **315**: 1519–1524.
42 Dillon JS. Dehydroepiandrosterone, dehydroepiandrosterone sulfate and related steroids: their role in inflammatory, allergic and immunological disorders. *Curr Drug Targets Inflamm Allergy* 2005; **4**: 377–385.

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ORIGINAL ARTICLE

Relationship between interleukin-6 and cerebral deep white matter and periventricular hyperintensity in elderly women

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Aim: We evaluated the relationships between serum levels of high-sensitivity C-reactive protein (hsCRP) and interleukin (IL)-6 with the severity of leukoaraiosis.

Methods: One hundred and thirty-seven elderly women who attended the Center for Comprehensive Care on Memory Disorders at Kyorin University Hospital were enrolled in this study. Leukoaraiosis was assessed by periventricular hyperintensity (PVH) score and deep white matter hyperintensity (DWMH) score.

Results: Serum log IL-6 level correlated with PVH and DWMH scores, but hsCRP did not. By multinomial logistic analysis, IL-6 was significantly related to DWMH score, independent of age and systolic blood pressure.

Conclusion: IL-6 is presumably an important marker of leukoaraiosis, as is the case with silent cerebral infarction. **Geriatr Gerontol Int 2011; 11: ••–••.**

Keywords: interleukin-6, leukoaraiosis, white matter hyperintensity.

Introduction

Leukoaraiosis, an isointense lesion on T_1-weighted images and hyperintense lesion on T_2-weighted images of magnetic resonance imaging (MRI), is considered to be a type of ischemic change in the brain on the basis of decreased blood flow in the area of leukoaraiosis.[1] In addition, leukoaraiosis is likely to have a relationship with vascular risk factors such as hypertension and diabetes.[2] On the other hand, the severity of leukoaraiosis also has a relationship with symptoms of the geriatric syndromes such as dementia, gait disturbance and functional disability.[3–5] Hence, leukoaraiosis is regarded as a significant brain lesion linking vascular risk factors and the occurrence of geriatric syndromes. Previous research on leukoaraiosis showed that women tended to have more white matter lesions than men,[6] and progression of deep white matter hyperintensity (DWMH) lesion was grater in women than men.[7] Furthermore, Gouw *et al.* showed that leukoaraiosis tended to develop greater in women than men and lacunes were vice versa.[8] Recently, many studies have focused on the relationships between brain ischemia and inflammation. Above all, Hoshi *et al.* demonstrated that serum high-sensitivity C-reactive protein (hsCRP) and interleukin (IL)-6 levels correlated with silent brain infarction.[9] They suggested an involvement of inflammation in cerebral infarction. However, few studies have examined the relationships between inflammatory markers and other cerebral ischemic changes such as leukoaraiosis.Therefore, we investigated whether serum levels of hsCRP and IL-6 have a relationship with leukoaraiosis in elderly women.

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Methods

Patients

One hundred and thirty-seven women who attended the Center for Comprehensive Care on Memory Disorders at Kyorin University Hospital were included in this study. This study was approved by the Ethics Committee of Kyorin University School of Medicine. Accordingly, written informed consent was obtained from all patients.

MRI

Magnetic resonance imaging (MRI) was performed on 1.5-T scanners (Toshiba Medical Systems, Tochigi, Japan). T_1-weighted images (repetition time [TR] = 496 msec, echo time [TE] = 12 msec), T_2-weighted images (TR = 4280 msec, TE = 105 msec) and fluid attenuated inversion recovery-weighted images (TR = 8000 msec, TE = 105 msec, 5 mm slice thickness) were obtained in the axial planes.

Periventricular hyperintensity and DWMH Score

Leukoaraiosis was classified as periventricular hyperintensity (PVH) adjacent to the lateral ventricle, and DWMH located in the deep white matter apart from the lateral ventricles. PVH was evaluated in six regions in three slices. Each region was rated as five grades (0–4) according to the systematic quantification method developed by Junque *et al.*[3] The sum of all grades in the six regions was defined as the PVH score (range 0–40).[4] DWMH was evaluated in the frontal, temporal, parietal and occipital lobes and in the basal ganglia in both hemispheres. Each lesion was rated as three grades according to the diameter, as described by de Groot *et al.*[5] The sum of all grades in five regions in both hemispheres was defined as the DWMH score.[4]

Laboratory tests

Blood samples were obtained in the morning after an overnight fast. Serum levels of hsCRP and IL-6 were measured using nephelometry and enzyme-linked immunosorbent assay, respectively. The intra-assay coefficients of variation for the measurements of hsCRP and IL-6 were 1.3% and 2.9%, respectively.

Statistical analysis

Because the distribution of hsCRP and IL-6 levels appeared to be left-skewed, they were normalized by logarithmic transformation. We used Spearman's ρ to investigate correlations between parameters and PVH score or DWMH score. Also, to test independently the effect of the inflammatory markers associated with the severity of leukoaraiosis, multinomial logistic regression analysis was performed with the grade of PVH (tertiles of PVH score) or DWMH (tertiles of DWMH score) as the dependent variable; and hsCRP or IL-6, together with age and systolic blood pressure (SBP) as independent variables. $P < 0.05$ was considered statistically significant. All data were analyzed using SPSS ver. 17.0.

Results

The characteristics of the study subjects are shown in Table 1. They were non-obese normolipidemic elderly persons, however, SBP was elevated. The distribution of PVH score and DWMH score of these subjects were 1–24 and 0–209, respectively. In Spearman's correlation coefficient, IL-6 correlated with PVH score ($\rho = 0.340$, $P \leq 0.05$) and DWMH score ($\rho = 0.299$, $P \leq 0.05$) (Fig. 1), whereas hsCRP showed no relation to PVH score or DWMH score (Table 2). PVH score and DWMH score also correlated with age and SBP. When log IL-6 and log hsCRP were grouped by tertile (see legend to Fig. 2), it was found that the average PVH score and DWMH score were higher in the highest tertile of IL-6 level than in the lowest tertile according to the Kruskal–Wallis test (Fig. 2a,b). On the other hand, this increment was not found in hsCRP (Fig. 2c,d).

Because leukoaraiosis can be observed on MRI even in normal elderly persons,[10] and hypertension is known to be a risk factor for leukoaraiosis,[11] we performed multinomial logistic regression analysis using PVH or DWMH severity (tertiles of PVH and DWMH score) as the dependent variable, and age, SBP and inflammatory

Table 1 Clinical characteristics of study subjects (women, $n = 137$)

Age (years)	76 ± 7
BMI (kg/m^2)	20.8 ± 3.3
SBP (mmHg)	142 ± 26
DBP (mmHg)	80 ± 14
PVH score (points)	8.2 ± 4.0
DWMH score (points)	61.4 ± 51.0
Total cholesterol (mmol/L)	5.38 ± 0.91
HDL cholesterol (mmol/L)	1.50 ± 0.36
LDL cholesterol (mmol/L)	3.23 ± 0.65
Triglyceride (mmol/L)	1.08 ± 0.46
Log IL-6 (ng/L)	0.35 ± 0.46
Log hsCRP (μg/L)	2.58 ± 0.58

All parameters are expressed as mean ± standard deviation. IL-6 and CRP are shown as log transformed. BMI, body mass index; DBP, diastolic blood pressure; DWMH, deep white matter hyperintensity; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; SBP, systolic blood pressure.

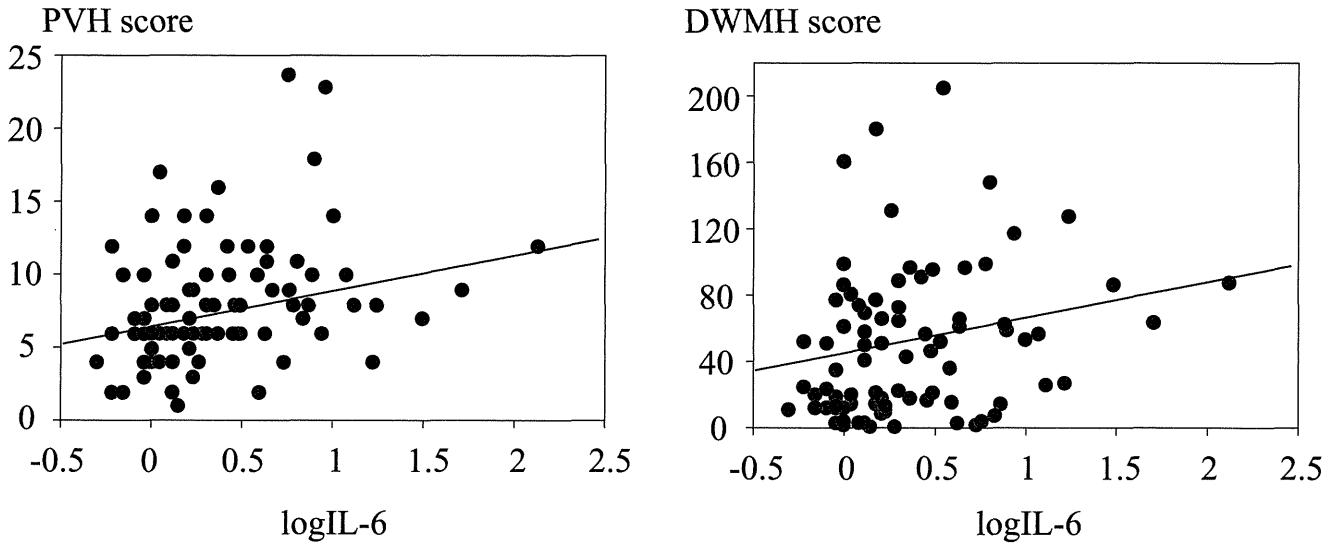

Figure 1 Relations between periventricular hyperintensity (PVH) score and log interleukin (IL)-6 (left panel; $\rho = 0.340$, $P \leq 0.05$, $n = 137$), and deep white matter hyperintensity (DWMH) score and log IL-6 (right panel; $\rho = 0.299$, $P \leq 0.05$, $n = 137$).

Table 2 Spearman's correlation coefficient between leukoaraiosis and parameters

	PVH score		DWMH score	
	ρ	*P*	ρ	*P*
Age	0.411	<0.001	0.271	0.002
BMI	−0.156	0.085	−0.124	0.179
SBP	0.215	0.014	0.232	0.009
Total cholesterol	−0.128	0.192	−0.149	0.134
HDL cholesterol	−0.053	0.595	−0.205	0.041
LDL cholesterol	−0.093	0.349	−0.025	0.802
Triglyceride	−0.014	0.885	0.080	0.421
Smoke	0.337	0.005	0.443	0.000
Log IL-6	0.340	0.002	0.299	0.006
Log hsCRP	−0.018	0.867	0.019	0.855

BMI, body mass index; DBP, diastolic blood pressure; DWMH, deep white matter hyperintensity; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; SBP, systolic blood pressure.

markers as independent variables. As shown in Table 3, it was confirmed that the level of IL-6 was significantly associated with the progression of PVH grade (from lowest to middle and middle to highest) and DWMH score (from middle to highest). However, this trend was not found in hsCRP.

Discussion

In this study, we showed relationships between IL-6 and PVH score and IL-6 and DWMH score. It is assumed that IL-6 has an association with cerebral ischemic changes such as leukoaraiosis as well as silent brain infarction.[9] Additionally, PVH and DWMH were correlated with IL-6, but not with hsCRP. With respect to this point, Schmidt *et al.* suggested that CRP is a marker of active carotid atherosclerosis, but not of a small vessel disease-related brain lesion.[12] On the other hand, it is envisaged that elevated hsCRP levels generally reflect large vessel atherosclerosis. Because leukoaraiosis is regarded as one of the brain changes caused by small vessel disease, our results support the idea of Schmidt *et al.*

Interleukin-6 is one of the principal acute-phase reactants, playing a significant role in the activation of the coagulation–fibrinolysis system. On the other hand, leukoaraiosis has been associated with a hypercoagulable condition. Endothelium-derived adhesion molecules have been reported to be elevated in patients with great leukoaraiosis or lacunar infarcts. Leukocyte-mediated injury of the small vessels and ensuing upregulation of endothelial adhesion molecules are implicated in the pathogenesis of leukoaraiosis.[13]

The Rotterdam Scan Study showed that higher hsCRP levels were associated with presence and progression of leukoaraiosis after adjustment for cardiovascular risk factors and carotid atherosclerosis.[14] The subjects in the Rotterdam Scan Study were a population-based cohort ($n = 1033$), while the subjects in the present study were outpatients in the memory clinic ($n = 137$). In this respect, the difference in characteristics and numbers of the subjects may have given rise to the different results in terms of hsCRP in the present study and the Rotterdam Scan Study.

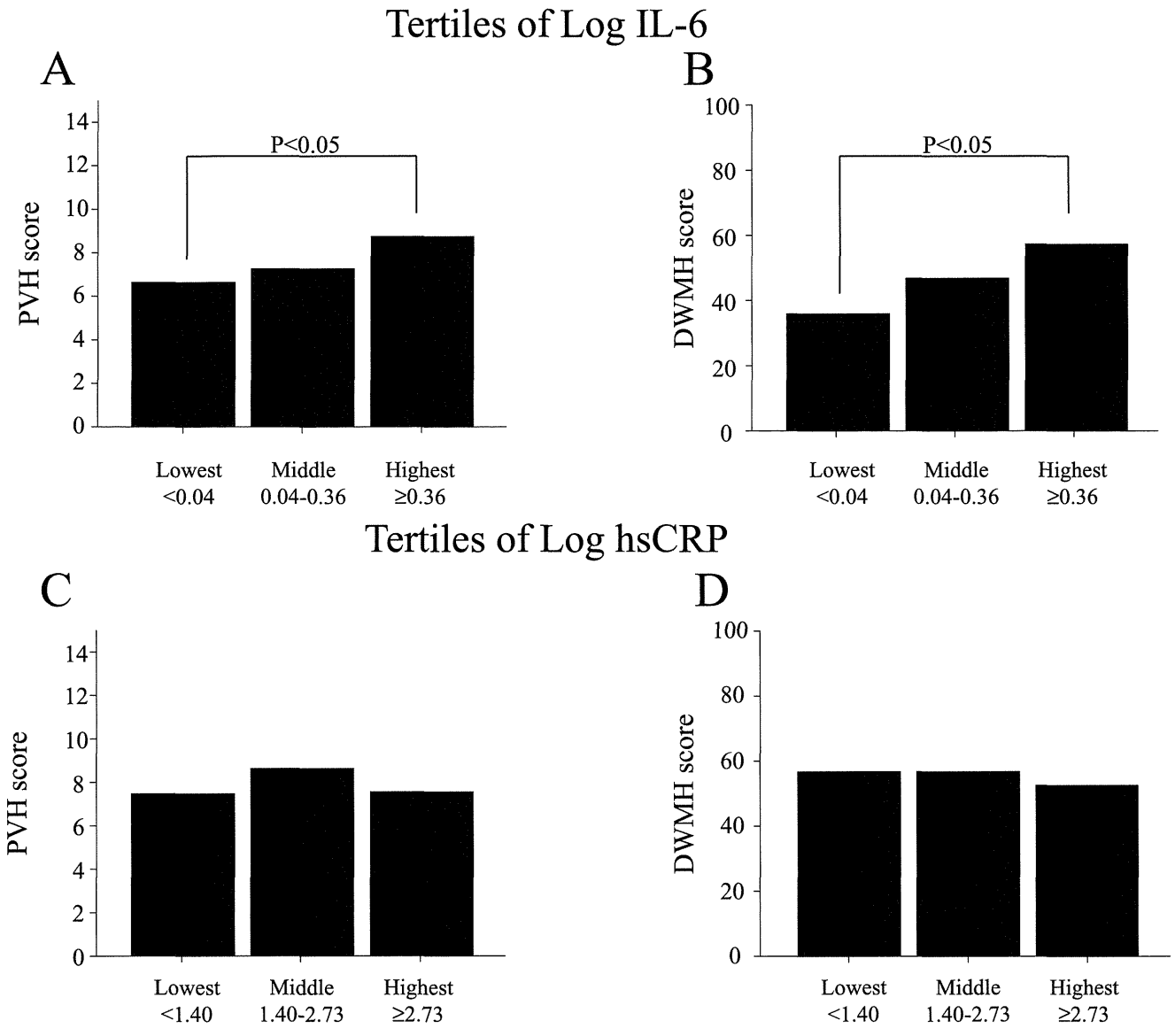

Figure 2 Average of periventricular hyperintensity (PVH) score and deep white matter hyperintensity (DWMH) score by tertile of interleukin (IL)-6 (a,b) and tertile of high-sensitivity C-reactive protein (hsCRP) (c,d). Log IL-6 tertile; lowest, <0.04 pg/mL, $n = 55$, 73.4 ± 7 1 years old (y/o); middle, 0.04–0.36 pg/mL, $n = 38$, 76.9 ± 6.8 y/o; highest, ≥0.36 pg/mL, $n = 44$, 79.5 ± 5.3 y/o. Log hsCRP; lowest, <1.40 ng/mL, $n = 44$, 73.9 ± 7.0 y/o; middle, 1.40–2.73 ng/mL, $n = 46$, 77.6 ± 7.1 y/o; highest, ≥2 73 ng/mL, $n = 41$, 77.8 ± 6.3 y/o.

In the Framingham Heart Study, no association was found between hsCRP and leukoaraiosis on MRI.[15] In the Cardiovascular Health Study, hsCRP level was modestly associated with semi-quantified leukoaraiosis volume, but the effect attenuated after excluding prevalent cerebrovascular and coronary disease cases.[13] In addition, Wright *et al.* was not able to find an association between hsCRP and leukoaraiosis volume.[16] Together, the relationships between leukoaraiosis and hsCRP varied depending upon different reports. This may come from the difference in study subjects and analytical methods. Further investigation is necessary to hold more definite opinion about which inflammatory biomarker represents the presence and development of leukoaraiosis.

Several lines of evidence suggest a relationship between IL-6 and symptoms of the geriatric syndromes, unique features of common health problems associated with poor morbidity in elderly people, such as dementia,[17] functional disability[18] and frailty.[19] On the other hand, the severity of leukoaraiosis also has a relationship with symptoms of geriatric syndromes such as dementia, falls, gait disturbance and functional disability.[3–5] Therefore, IL-6 may be an important biomarker linking the severity of leukoaraiosis to the geriatric syndromes. Because the present study is

Table 3 Associations between inflammation markers and the severity of leukoaraiosis according to tertiles (PVH score or DWMH score) adjusting for age and systolic blood pressure (logistic regression analysis)

	Log hsCRP, μg/L Odds ratio (95% CI)	Log IL-6, ng/L Odds ratio (95% CI)
PVH grade (tertiles)		
Lowest to middle	1.84 (0.78–4.31)	5.80 (1.43–23.60)
Middle to highest	0.39 (0.12–1.32)	4.39 (1.02–18.85)
DWMH grade (tertiles)		
Lowest to middle	0.81 (0.333–1.99)	3.18 (0.78–12.95)
Middle to highest	1.25 (0.48–3.29)	7.85 (1.69–36.38)

Grade of leukoaraiosis according to tertiles of PVH score or DWMH score. CI, confidence interval; DWMH, deep white matter hyperintensity; IL-6, interleukin-6; hsCRP, high-sensitivity C-reactive protein; PVH, periventricular hyperintensity.

cross-sectional, a longitudinal study would corroborate the associations of IL-6 with leukoaraiosis, and IL-6 with the geriatric syndromes.

In conclusion, we demonstrated that IL-6 level is significantly associated with the severity of PVH and DWMH lesions. The results of the present study, together with the previous studies, suggest that IL-6 is an important marker of the progression of cerebral ischemic disease, linking to the presence of geriatric syndromes.

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References

1 Oishi M, Mochizuki Y. Regional cerebral blood flow and cerebrospinal fluid glutamate in leukoaraiosis. *J Neurol* 1998; **245**: 777–780.
2 Munoz DG. Leukoaraiosis and ischemia: beyond the myth. *Stroke* 2006; **37**: 1348–1349.
3 Junque C, Pujol J, Vendrell P *et al.* Leuko-araiosis on magnetic resonance imaging and speed of mental processing. *Arch Neurol* 1990; **47**: 151–156.
4 Sonohara K, Kozaki K, Akishita M *et al.* White matter lesions as a feature of cognitive impairment, low vitality, and other symptoms of the geriatric syndrome in the elderly. *Geriatr Gerontol Int* 2008; **8**: 93–100.
5 de Groot JC, de Leeuw FE, Oudkerk M *et al.* Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann Neurol* 2000; **47**: 145–151.
6 de Leeuw FE, de Groot JC, Achten E *et al.* Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatry* 2001; **70**: 9–14.
7 van den Heuvel DM, Admiraal-Behloul F, ten Dam VH *et al.* Different progression rates for deep white matter hyperintensities in elderly men and women. *Neurology* 2004; **63**: 1699–1701.
8 Gouw AA, van der Flier WM, Fazekas F *et al.* Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke* 2008; **39**: 1414–1420.
9 Hoshi T, Kitagawa K, Yamagami H *et al.* Relations of serum high-sensitivity C-reactive protein and interleukin-6 levels with silent brain infarction. *Stroke* 2005; **36**: 768–772.
10 Fazekas F. Magnetic resonance signal abnormalities in asymptomatic individuals: their incidence and functional correlates. *Eur Neurol* 1989; **29**: 164–168.
11 Basile AM, Pantoni L, Pracucci G *et al.* Age, hypertension, and lacunar stroke are the major determinants of the severity of age-related white matter changes. The LADIS (Leukoaraiosis and Disability in the Elderly) Study. *Cerebrovasc Dis* 2006; **21**: 315–322.
12 Schmidt R, Schmidt H, Pichler M *et al.* C-reactive protein, carotid atherosclerosis, and cerebral small-vessel disease: results of the Austrian Stroke Prevention Study. *Stroke* 2006; **37**: 2910–2916.
13 Fornage M, Chiang YA, O'Meara ES *et al.* Biomarkers of inflammation and MRI-defined small vessel disease of the brain: the cardiovascular health study. *Stroke* 2008; **39**: 1952–1959.
14 van Dijk EJ, Prins ND, Vermeer SE *et al.* C-reactive protein and cerebral small-vessel disease: the Rotterdam Scan Study. *Circulation* 2005; **112**: 900–905.
15 Jefferson AL, Massaro JM, Wolf PA *et al.* Inflammatory biomarkers are associated with total brain volume: the Framingham Heart Study. *Neurology* 2007; **68**: 1032–1038.
16 Wright CB, Moon Y, Paik MC *et al.* Inflammatory biomarkers of vascular risk as correlates of leukoariosis. *Stroke* 2009; **40**: 3466–3471.
17 Dziedzic T. Systemic inflammatory markers and risk of dementia. *Am J Alzheimers Dis Other Demen* 2006; **21**: 258–262.
18 Ferrucci L, Harris TB, Guralnik JM *et al.* Serum IL-6 level and the development of disability in older persons. *J Am Geriatric Soc* 1999; **47**: 639–646.
19 Leng S, Chaves P, Koenig K, Walston J. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. *J Am Geriatric Soc* 2002; **50**: 1268–1271.

Geriatr Gerontol Int 2011; **11:** *3–7*

COMMISSION REPORT

Survey on geriatricians' experiences of adverse drug reactions caused by potentially inappropriate medications: Commission report of the Japan Geriatrics Society

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Aim: The Japan Geriatrics Society (JGS) developed the guidelines for medical treatment and its safety in the elderly and the list of potentially inappropriate medication use, a Japanese version of the Beers list, in 2005. The JGS working group in collaboration with the Japan Broadcasting Corporation conducted the survey to geriatricians to investigate their experiences of adverse drug reactions (ADR) caused by potentially inappropriate medications.

Methods: In September 2008, the survey mails were sent to all the JGS certified geriatricians ($n = 1492$). The questionnaire consisted of 1 year of experiences of ADR of any type, past experiences of ADR by the use of antipsychotic benzamides, hypnotic benzodiazepines, digoxin (≥0.15 mg/day), vitamin D_3 (alfacalcidol ≥1.0 μg/day) and additional drugs, and their attitudes to reduce the dose/number of drugs for the prevention of ADR.

Results: A total of 425 geriatricians responded (response rate 29%). Seventy-two percent experienced ADR within 1 year. Past experiences of ADR were reported by 79% for antipsychotic benzamides, 86% for hypnotic benzodiazepines, 70% for digoxin and 37% for vitamin D_3. Free responses included frequent ADR by non-steroidal anti-inflammatory, antihypertensive, antiplatelet, anti-arrhythmic, antidiabetic and antidepressant drugs. Reduction of drugs for ADR prevention was attempted by 93%.

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Conclusion: This survey showed that most geriatricians experience ADR and take preventive measures for ADR. The results can be used for education and the development of new guidelines. **Geriatr Gerontol Int 2011; 11: 3–7.**

Keywords: adverse drug reactions, Beers list, geriatrician, polypharmacy, side-effect.

Introduction

Adverse drug reactions (ADR) are more frequent and severe in the elderly than in young adults. A recent systematic review[1] of prospective observational studies reported that 10.7% of hospital admissions were associated with ADR in elderly patients, while 6.3% were so in young adults. Surveys performed in acute care hospitals in Japan also showed that inpatients aged 70 years or older were 1.5-fold more likely to develop ADR than those under 60 years of age,[2] and that the ADR incidence among elderly inpatients was 6–15%.[3] It has been reported from Western countries that ADR occur in more than 10% of outpatients or nursing home residents.[4]

Although many factors relate to the high ADR incidence in the elderly, overdoses resulting from age-related changes of pharmacokinetics/pharmacodynamics and polypharmacy may be of critical importance.[2–4] Because the evidence for the elderly is limited, practical guidelines to medical treatment and its safety are required in the field of geriatric medicine.

The Japan Geriatrics Society (JGS) has conducted educational activities through scientific sessions and official journals to reduce ADR. As part of activities, the ad hoc committee "Working group on guidelines for medical treatment and its safety in the elderly" was set up in 2003, and the JGS guidelines for medical treatment and its safety in the elderly were published in 2005.[5] In the guidelines, the list of medications that should be prescribed with special attention to elderly patients was reported and was put on the JGS website. This list, a Japanese version of the Beers list,[6,7] consists of 45 drugs or drug classes that may be harmful or less efficient, thus potentially inappropriate for elderly patients, and can be applied to reduce ADR and polypharmacy in clinical settings of geriatric medicine and nursing-care facilities.[5]

Although the mass media expressed an interest in these activities, the JGS should increasingly accumulate the evidence and make a proposal on pharmacotherapy of the elderly for public education. For this purpose, the JGS working group in collaboration with the Japan Broadcasting Corporation (NHK) conducted the survey to JGS certified geriatricians to investigate their experiences of ADR caused by potentially inappropriate medications. This commission report of the working group shows the survey results.

Methods

Mailing and collection of the questionnaire

In September 2008, the questionnaire was mailed by the NHK to all the JGS certified geriatricians ($n = 1492$) who appeared on the JGS website. In the cover letter, a brief introduction including the background and aim of the survey was described, followed by the statement that this survey was carried out in collaboration with the NHK and the JGS working group. The JGS version of the Beers list (Table 1 and detailed explanation) was included in the mail for options of additional drugs. The responder was asked to return the questionnaire to the NHK by fax without his/her name.

Questionnaire item

The questionnaire consisted of 1-year experiences of ADR of any type (yes/no question), past experiences (frequent, occasional or none) of ADR by the use of antipsychotic benzamides (sulpiride, sultopride), hypnotic benzodiazepines (flurazepam, haloxazolam, quazepam, triaxolam), digoxin (≥0.15 mg/day), vitamin D_3 (alfacalcidol ≥1.0 μg/day) and free additions, and their attitudes to reduce the dose/number of drugs for the prevention of ADR (yes/no question). In addition, free comments on the problems and approaches related to pharmacotherapy in the elderly were asked. The above four classes of drugs were chosen from the JGS version of the Beers list (Table 1) because these drugs were considered frequently prescribed to elderly patients.

Statistical analysis

The data are shown as the number and the percent of subjects. The χ^2-test was performed to analyze the associations between ADR experiences.

Results

A total of 425 geriatricians responded, resulting in a response rate of 28.5%. The response rate would have been 29.1% if the 30 subjects to whom the mails were not successfully delivered were excluded.

The summary of the results is shown in Table 2. Seventy percent of the geriatricians reported