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What Is Oxidative Stress?

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Abstract: Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies. Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA. However, oxidative stress also has a useful role in physiologic adaptation and in the regulation of intracellular signal transduction. Therefore, a more useful definition of oxidative stress may be "a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them." The biomarkers that can be used to assess oxidative stress *in vivo* have been attracting interest because the accurate measurement of such stress is necessary for investigation of its role in lifestyle diseases as well as to evaluate the efficacy of treatment. Many markers of oxidative stress have been proposed, including lipid hydroperoxides, 4-hydroxynonenal, isoprostan, 8-hydroxyguanine, and ubiquinol-10. To prevent the development of lifestyle diseases, advice on how to lead a healthy life should be given to individuals based on the levels of oxidant and antioxidant activity assessed by pertinent biomarkers. Individual genetic information should also be taken into consideration.

Key words: Oxidative stress; Free radicals; Active oxygen; Biomarkers

Introduction

The close association between oxidative stress and lifestyle-related diseases has become well known. Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them." It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs.

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Table 1 Major Active Oxygen Species

$O_2^{\bullet -}$	Superoxide radical
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical
$^1O_2$	Singlet oxygen
$HOO^{\bullet}$	Hydroperoxyl radical
LOOH	Alkylhydroperoxide
$LOO^{\bullet}$	Alkylperoxyl radical
$LO^{\bullet}$	Alkoxyl radical
CIO^-	Hypochlorite ion
$Fe^{4+}O$	Ferryl ion
$Fe^{5+}O$	Periferryl ion
$NO^{\bullet}$	Nitric oxide

Free Radicals, Active Oxygen Species, and Oxidative Stress

Usually, an atom is composed of a central nucleus with pairs of electrons orbiting around it. However, some atoms and molecules have unpaired electrons and these are called free radicals. Free radicals are usually unstable and highly reactive because the unpaired electrons tend to form pairs with other electrons. An oxygen molecule (O_2) undergoes four-electron reduction when it is metabolized *in vivo*. During this process, reactive oxygen metabolites are generated by the excitation of electrons secondary to addition of energy or interaction with transition elements. The reactive oxygen metabolites thus produced are more highly reactive than the original oxygen molecule and are called active oxygen species. Superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen are active oxygen species in the narrow sense. Active oxygen species in a broad sense are listed in Table 1. Only active oxygen species having an unpaired electron, indicated with a dot above and to the right of the chemical formula in the table, are free radicals.

For aerobic organisms, a mechanism to remove these highly reactive active oxygen species is essential to sustain life. Therefore, various antioxidant defense mechanisms have developed in the process of evolution. It is also true that the high reactivity of these oxygen metabolites is utilized to control various biological phenomena.

From a biological viewpoint, various oxygen-derived free radicals have been attracting attention for the following reasons: Various active oxygen species are generated in the body during the process of utilizing of oxygen. Because the body is furnished with elaborate mechanisms to remove active oxygen species and free radicals, these by-products of oxygen metabolism are not necessarily a threat to the body under physiological conditions. However, if active oxygen species or free radicals are generated excessively or at abnormal sites, the balance between formation and removal is lost, resulting in oxidative stress. Consequently, active oxygen species and free radicals can attack molecules in biological membranes and tissues, thus inducing various diseases. In other words, oxidative stress is defined as a "state harmful to the body, which arises when oxidative reactions exceed antioxidant reactions because the balance between them has been lost."

However, oxidative stress is actually useful in some instances. For example, oxidative stress induces apoptosis to prepare the birth canal for delivery. Also, biological defense mechanisms are strengthened by oxidative stress during appropriate physical exercise and ischemia. Therefore, a more useful definition of oxidative stress may be a "state where oxidation exceeds the antioxidant systems because the balance between them has been lost."

Biomarkers of Oxidative Stress

The biomarkers that can be used to assess oxidative stress have been attracting interest because the accurate assessment of such stress is necessary for investigation of various pathological conditions, as well as to evaluate the efficacy of drugs. Assessment of the extent of oxidative stress using biomarkers is interesting from a clinical standpoint. The markers found

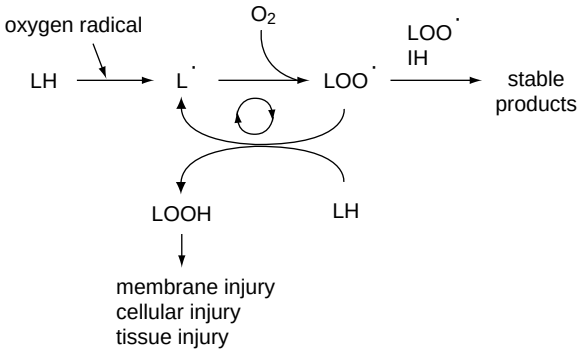


Fig. 1 The chain reaction causing lipid peroxidation

in blood, urine, and other biological fluids may provide information of diagnostic value, but it would be ideal if organs and tissues suffering from oxidative stress could be imaged in a manner similar to CT scanning and MR imaging. In recent years, attempts have been made to use electron spin resonance techniques for this purpose, but it will take time before such methods can be applied to humans.

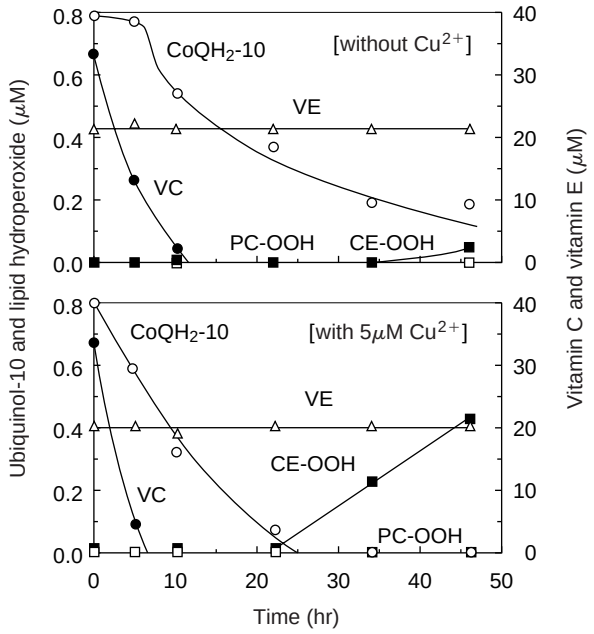
Because the body is not necessarily fully protected against oxidative damage, some of its constituents may be injured by free radicals, and the resultant oxidative products have usually been used as markers. Many markers have been proposed, including lipid peroxides, malondialdehyde, and 4-hydroxynonenal as markers for oxidative damage to lipids; isoprostan as a product of the free radical oxidation of arachidonic acid; 8-oxoguanine (8-hydroxyguanine) and thymineglycol as indicators of oxidative damage to DNA; and various products of the oxidation of protein and amino acids including carbonyl protein, hydroxyleucine, hydrovaline, and nitrotyrosine. Lipid peroxide was assessed in clinical samples even in relatively early studies, and the analytical methods for this substance have improved.

The famous method of Yagi, which measures substances that react with thiobarbituric acid, has been widely used in both clinical and experimental studies. Such substances have been the most frequently used marker of oxidative stress partly because lipid peroxidation (Fig. 1) is a very important mechanism of cell membrane destruction. Lipid peroxidation is a chain reaction by which unsaturated fatty acids (cell membrane components) are oxidized in various pathological conditions.

When a hydrogen atom is removed from a fatty acid molecule for some reason, the free radical chain reaction proceeds as shown in Fig. 1. Thus, radicals that can be involved in the extraction of hydrogen atoms from lipids include the hydroxyl radical ($HO^{\bullet}$), the hydroperoxyl radical ($HOO^{\bullet}$), the lipid peroxyl radical ($LOO^{\bullet}$), and the alkoxyl radical ($LO^{\bullet}$). Metal-oxygen complexes, particularly iron-oxygen complexes, are also important *in vivo*. The peroxidation chain reaction propagates itself once it has started. The process by which lipid radicals ($L^{\bullet}$) are generated from lipids (LH) is called the chain initiation reaction. Lipid radicals ($L^{\bullet}$) thus generated react immediately with oxygen, resulting in the formation of $LOO^{\bullet}$, which attacks another lipid and removes a hydrogen atom from it, resulting in the formation of lipid hydroperoxide (lipid peroxide; LOOH) and another $L^{\bullet}$. This new $L^{\bullet}$ also reacts with oxygen and forms $LOO^{\bullet}$, which attacks another lipid to generate lipid peroxide, so lipid peroxide accumulates as the chain reaction proceeds.

Gastric mucosal injury occurs in patients with extensive burns. Before the development of mucosal lesions, the blood level of skin-derived substances that react with thiobarbituric acid shows an increase. Then these substances also increase in the gastric mucosa, leading to the development of mucosal lesions. The free radical peroxidation of lipids is an important factor in local injury to cell membranes and impairment of the activity of enzymes and receptors bound to the membrane, and the lipid peroxide thus produced can affect even remote organs.

Among the agents that protect the body from lipid peroxidation, vitamin E is consid-



$CoQH_2$-10; coenzymeQ_{10}
VC; vitamin C, VE; vitamin E
PC-OOH; phosphatidylcholine hydroperoxide
CE-OOH; cholesterylester hydroperoxide

Fig. 2 Changes of antioxidants and generation of lipid peroxides during incubation of human plasma at 37°C in air

Source: Yamamoto, Y. *et al.*: *Oxidative Damage and Repair*. ed. Davies, K.J.A., Pergamon Press, 1991; pp. 287–291.

ered to be the most important. This vitamin has attracted attention as an antioxidant because it can scavenge lipid peroxyl radicals and hence stop the propagation of the free radical chain reaction. The lipid peroxyl radical removes a hydrogen atom from the phenyl group of vitamin E and the molecule that has accepted the hydrogen atom is stabilized. In turn, vitamin E is converted into a radical, which is also stable and less reactive. Consequently, this vitamin E-derived radical is unlikely to attack lipids and perpetuate the chain reaction. Instead, it is thought to react with another peroxyl radical and thus become stable. This antioxidant reaction protects biological membranes from injury caused by free radicals and lipid peroxides.

However, lipid peroxides are still generated in the plasma despite the presence of an adequate concentration of vitamin E. Consequently, plasma vitamin E levels seem unlikely to be a useful biomarker of oxidative stress. In addition, vitamin E is lipid soluble, so its blood level varies depending on the lipid content.

When human plasma is incubated at 37°C in air, the concentrations of antioxidants and lipid peroxides change as shown in Fig. 2. Of the three antioxidants, vitamin C decreases first, followed by reduced coenzyme Q-10 (ubiquinol-10). This suggests that vitamin C and ubiquinol-10 are the antioxidants that are most sensitive to oxidative stress. Vitamin E may be protected by vitamin C and ubiquinol-10 because it is an important antioxidant. Vitamin C and ubiquinol-10 levels were measured to assess oxidative stress in patients with various liver diseases. In patients with chronic hepatitis, liver chirrhosis, and liver cancer, the vitamin C and ubiquinone-10 (oxidized coenzyme Q-10) levels were significantly decreased and increased, respectively, when compared with those in the control group, with a significant percent increase of oxidized coenzyme Q-10. In contrast, there was no significant difference of the vitamin E level.

Oxidative Stress as a Biological Modulator and as a Signal (Fig. 3)

Oxidative stress not only has a cytotoxic effect, but also plays an important role in the modulation of messengers that regulate essential cell membrane functions, which are vital for survival. It affects the intracellular redox status, leading to the activation of protein kinases, including a series of receptor and non-receptor tyrosine kinases, protein kinase C, and the MAP kinase cascade, and hence induces various cellular responses. These protein kinases play an important role in cellular responses such as activation, proliferation, and differentiation, as well as various other functions. Accordingly, the protein kinases have attracted the most attention in the investigation of the association between oxidative stress and disease.

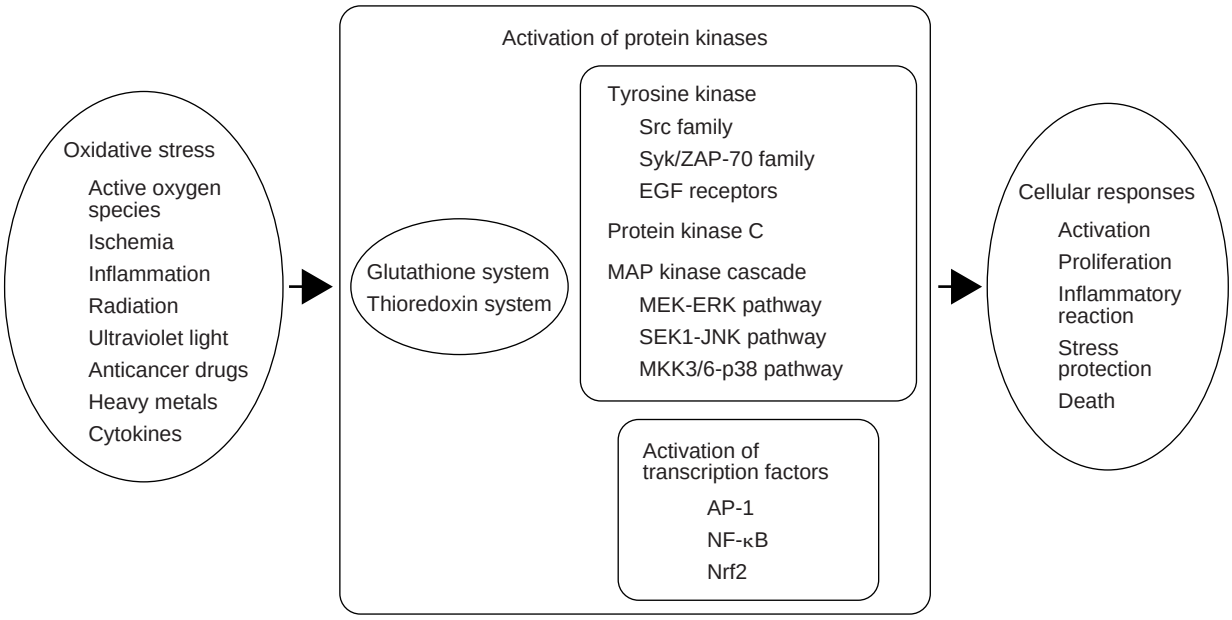


Fig. 3 Oxidative stress and cellular responses

Oxidative stress can influence many biological processes such as apoptosis, viral proliferation, and inflammatory reactions. In these processes, gene transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) act as oxidative stress sensors through their own oxidation and reduction cycling. This type of chemical modification of proteins by oxidation and reduction is called reduction-oxidation (redox) regulation.

The transcription factor NF-κB undergoes translocation from the cytoplasm to the nucleus in response to an extracellular signal. This translocation induces its ability to bind to DNA, leading to transcriptional up-regulation of the expression of many genes related to inflammation and immunity. Thus, NF-κB seems to be involved in development and aggravation of many diseases. Recently, it was also suggested that this factor may be involved in the process of carcinogenesis because it is located upstream to a series of transcription regulation factors and because it possesses the ability to suppress apoptosis.

With respect to the role that oxidative stress plays in the activation of NF-κB, many new findings have been obtained recently. Stimulation with tumor necrosis factor (TNF)-α, phorbol myristate acetate (PMA), interleukin (IL)-1, lipopolysaccharide, viral infection, and ultraviolet light leads to the generation of active oxygen species, which function as a second messenger in the activation of NF-κB. The mitochondrial respiratory chain is considered to be the major source of active oxygen species. In cells lacking mitochondria, damage caused by TNF-α and NF-κB dependent IL-6 production is suppressed. It has also been shown that antimycin A, an inhibitor of mitochondrial electron transport, increases the intracellular generation of active oxygen species and enhances the activation of NF-κB. In resting cells, NF-κB is bound to IκB and remains in the cytoplasm. An extracellular signal causes the dissociation of these two molecules and IκB decomposes, whereupon NF-κB migrates to the nucleus and activates transcription.

The phosphorylation cascade that produces the NF-κB/IκB complex has been shown to depend on the interaction between proteins

derived from activation of IL-1 and TNF receptors. The activation of NB-κB requires a signal derived from active oxygen species. The possible involvement of active oxygen species in the release of NF-κB is partly suggested because IκB undergoes phosphorylation via a group of kinases involved in a phosphorylation cascade. Induction of the expression of thioredoxin by active oxygen species is also involved in the activation of NF-κB, since thioredoxin gives NF-κB the ability to bind to DNA in a process that is regulated by redox reactions.

NF-κB seems to be the key transcription factor for elucidating the relationship of oxidative stress to lifestyle diseases and identification of the precise mechanisms involved may lead to the development of new therapies for such diseases.

Conclusion

The causes of lifestyle diseases can be divided into three major categories, which are genetic, habitual, and environmental. Many of the genes that are associated with biological oxidative stress have been identified, with the genes for NO synthetase (NOS) and heme oxygenase (HO) being considered as candidates for such diseases. However, lifestyle diseases are often multifactorial, so it is difficult to identify the causative factors. Recent progress in the field of molecular biology has made it possible to store massive amounts of genetic information on DNA microchips and has provided various efficient computer programs for analysis, thus promising rapid progress in this field.

Many daily habits are closely associated with oxidative stress, which is augmented by smoking, drinking, and an irregular diet. In Japan, dietary habits have undergone a marked change over the years. When the energy intake related to major nutrients is calculated, lipids provide over 25%, reflecting this change. Many environmental factors can generate active oxygen species and DNA damage caused by such oxygen radicals is extremely serious because it may be related to carcinogenesis. To prevent the development of lifestyle diseases, instructions on how to lead a healthy life should be given individually depending on the level of antioxidant activity assessed by pertinent biomarkers. Individual genetic information should also be taken into consideration when giving such instructions. Such health issues may become central to medical care in the 21st century.

REFERENCES

1) Yoshikawa, T.: *A Guide to Free Radicals*. Part 2. Sentan Igaku Sha, Tokyo, 1998.
2) Yoshikawa, T.: *Medicine of Free Radicals*. Shindan to Chiryo Sha, Tokyo, 1997.
3) Yoshikawa, T.: *Science of Free Radicals*. Koudan Sha Saientifikku, Tokyo, 1997.

Aging and Oxidative Stress

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Abstract: Research on the aging mechanism in the body and the regulation of cellular life has progressed owing to recent advances in molecular biology. Based on several lines of evidence, aging is considered as a biological process closely related to oxidative stress. While oxygen is indispensable to the life of the organism, it also causes oxidative stress. Since all of the organisms on earth evolved with this dilemma, it is important to further investigate the aging mechanism and control of oxidative stress.

Key words: Program hypothesis; Limited proliferation capability; Error catastrophe hypothesis; Oxidatively modified base

Introduction

Lifestyle-related diseases such as diabetes, hypertension, hyperlipidemia, and atherosclerosis develop in parallel with aging and are closely correlated to senescent change. Research on the aging mechanism in the body and the regulation of cellular life has made remarkable advances owing to recent molecular investigations. It is considered that the molecular mechanism for the proliferation capability of the somatic cell is related closely to the longevity of the organism. Thus far, the intrinsic programs, which originally exist in the organism, such as telomere shortening and limited proliferation capability of the somatic cell, as well as the external environments including oxidative stress causing error accumulation in biological information have been identified as representative factors to determine the life span of the organism. Although oxygen is an indispensable element contributing to the survival of aerobic organisms on the earth, it can also become a harmful factor to their lives. Therefore, oxygen could have two contradictory functions, namely acting as an essential element for maintenance of life and a toxic factor leading to cell damage and death. In the current article, we review the involvement of oxidative stress in the molecular mechanism of aging.

"The Life Span Clock" in the Organism: Hayflick's Limited Proliferation Capability Hypothesis of the Somatic Cell

In multicellular organisms such as human,

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aging process is characterized as a decline in organ function over time. There seems to be degeneration of tissues with a decrease in cell population, which could be closely related to senescent change of the cell. Thus far, many hypotheses on the cellular aging mechanism have been proposed. The program hypothesis and the error catastrophe hypothesis comprise two of the representative hypotheses.[1)] In the program hypothesis, population doubling level (PDL) is generally used as an index of capability of cell division. PDL differs in each species and shows a good correlation with the life span of the species. For instance, the PDL of the human somatic cell is approximately fifty. Based on this hypothesis, aging is regarded as a process destined by the specific program within the somatic cells that constitutes the organism. In 1965, Hayflick found that the somatic cells discontinue proliferation after they completed a fixed number of cycles. In other words, the human cultured cell repeats cell division as a means of proliferation, which stops once it reaches a certain number of division cycle. Hayflick defined the cycle number as the finite proliferation capability of the somatic cell, and considered that this phenomenon might reflect cell aging.[2)] Hayflick proposed that aging of the somatic cell proceeds according to a regulated time course, at the end of which the cells come to their death. He explained that this could control the life span of the individual.

Error Catastrophe Hypothesis as an External Factor Regulating Cell Death

Provided that the somatic cells have their own fatal life span, could the program hypothesis explain the aging process in every organism? If the program hypothesis is applied, pure line with identical genetic background animals should have the same life span. Nevertheless, in reality, the life span of these animals still depends on the external environment. Therefore, the program hypothesis cannot always explain the aging of the organisms. In order to account for this discordance, the error catastrophe hypothesis was proposed as an alternative. According to this hypothesis, it is speculated that, genetic mutations caused by environmental factors play important roles. Integrated cytogenetic changes stop cell division, thereby allowing the individual to advance in age.

However, the fixed PDL of each species cannot be explained sufficiently by the catastrophe hypothesis. Furthermore, the catastrophe hypothesis was not able to interpret the observations in the cell fusion experiments using the cell lines with different life spans. When the cell of a short life span was fused with that of a long life span, the cell produced by the fusion should have a long life span by mutual supplement of the defect. However, those cells produced by such cell fusion usually have a short life span. In addition, when genetic information of the aged cell is transferred into the infinite proliferated cell line, cell division is terminated. Therefore, from the viewpoint of cell division control, external factor and intrinsic factor seem to interact with each other for aging: the former acts as a damage factor for DNA; and the letter as genetic characteristics for replication control.[1,3)]

The Telomere and Telomerase

The program hypothesis interprets cell division capability as being controlled by intrinsic factors. The telomere, the terminal structure of a chromosome, plays an important role in this mechanism. The telomere has a tandem repetitive sequence of 6 bases, TTAGGG, in each species and can function to prevent gene disruption near the end of the chromosome during cell division and DNA replication. It seems that the telomere protects the cell from misdivision through shortening.

The following interpretation is presented as an explanation of this mechanism. The somatic cell uses RNA to prime DNA synthesis, synthesizing DNA from the 5'side of the base

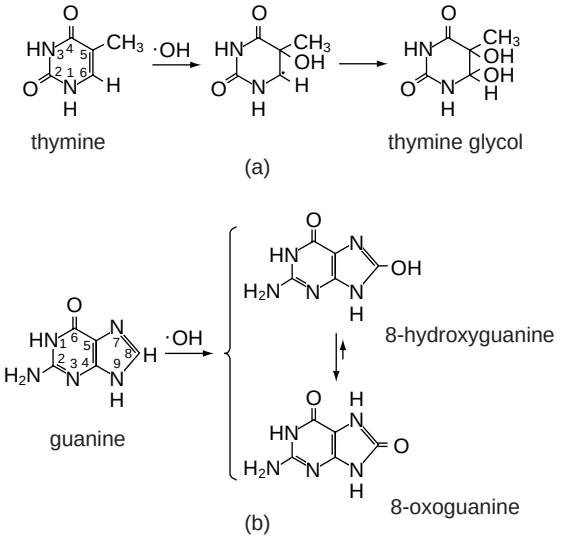

Fig 1 Formation process of the modified base (quoted from reference 3)

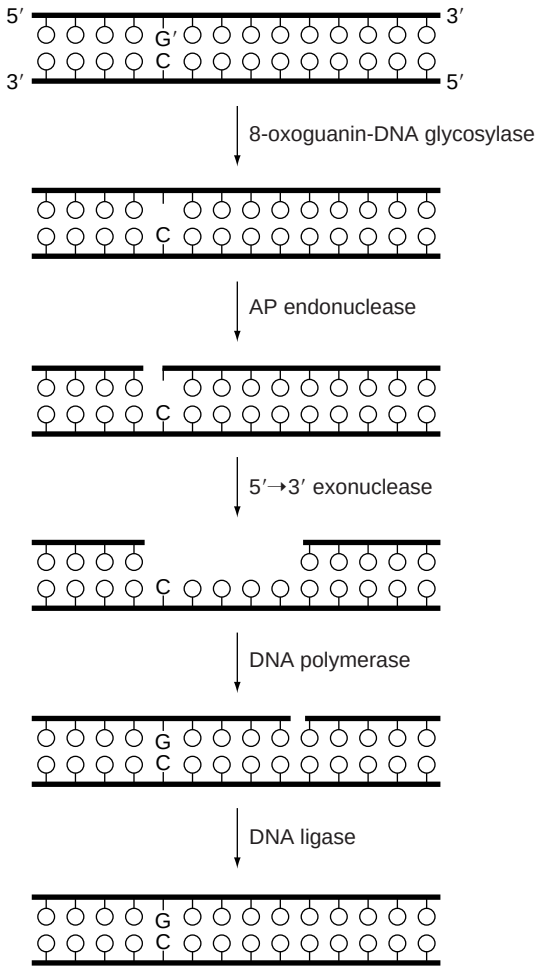

Fig. 2 Repair mechanism in DNA injured by 8-hydroxyguanine (shown as G', quoted from reference 3)

sequence, and the replicated DNA chain is shortened by the RNA size.[4,5] Actually, the telomere shortens rectilinearly in every cell division. To protect from telomere shortening, an enzyme designated as telomerase has been reported and attracted many researchers' attention. This enzyme is a complex consisting of the p80 subunit of 80 kDa, the p95 of 95 kDa and RNA as the template of DNA replication. Telomerase is known to restore the replicated DNA sequences, thereby protecting the telomere structure.[6]

The Relationship between Oxidative Stress and Cellular Aging

Based on these findings, endogenous factors including the telomere seem to play a regulatory role in the aging process, coordinated with various external factors presumably modifying the endogenous factors. Of such external factors, active oxygens have greatly attracted the researchers' attention.

The following 4 types of molecules are characteristic active oxygens: hydrogen peroxide (H_2O_2), superoxide anion ($\cdot O_2^-$), hydroxyl radical ($\cdot$OH), and singlet oxygen ($^1O_2$). Active oxygen molecules are always generated when the organism respires in an aerobic environment.[3] For example, superoxide anion is formed in reactions catalyzed by NADPH oxidase as well as when an excess electron (e^-) from the physiological metabolism is provided to oxygen (O_2). The superoxide anion either reacts with oxidized cytochrome c or is catalyzed by superoxide dismutase (SOD) to form hydrogen peroxide. Hydrogen peroxide reacts specifically with catalase and peroxidase and is then removed, while hydrogen peroxide forms a hydroxyl radical in the presence of an iron ion.

As the reactivity of hydrogen peroxide and superoxide anion is very high, these materials

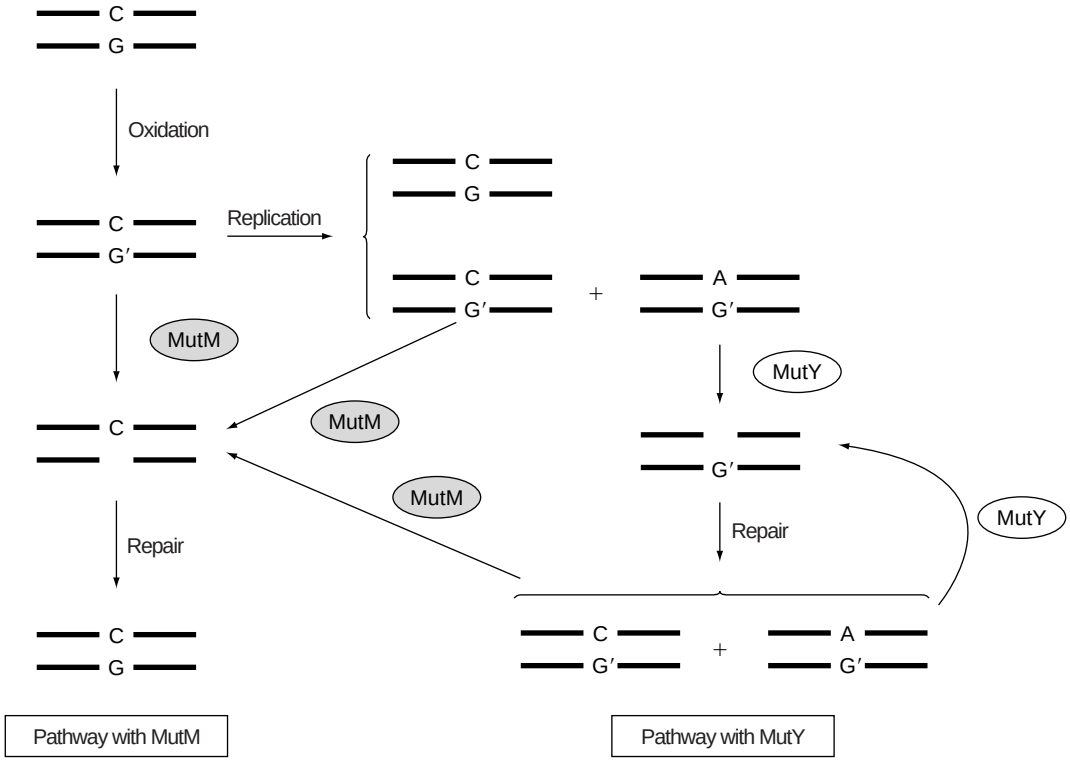

Fig. 3 Role of MutM and MutY in DNA injured by 8-hydroxyguanine (shown as G', quoted from reference 3)

damage various molecules in the organism. The target elements include cellular proteins, the biological membranes, nucleic acids, and organelles such as the endoplasmic reticulum. Among those biological targets, the injury to the DNA chain causes biological error related to the life span of the cell.

The target in the DNA chain includes deoxyribose and base parts, etc. When the DNA chain is damaged, guanine is converted to 8-hydroxy guanine and thymine is modified to thymine glycol.[7,8] The conversion to 8-hydroxy guanine is shown in Fig. 1.

The modified base is removed in the successive reaction by the following enzymes: 8-oxoguanine-DNA glycosylase, AP (apurinic/apyrimidinic) endonuclease, exonuclease, DNA polymerase, and DNA ligase (Fig. 2).

Among those enzymes, 8-oxoguanine DNA glycosylase is the counterpart of MutM, which is abundantly expressed in aerobacteria, and involved in removal of the modified base.[9] The formed apurinic site or apyrimidinic site is catalyzed by AP endonuclease, and then the damaged site is repaired by exonuclease, DNA polymerase, and DNA ligase. The resulting 8-hydroxy guanosine is discharged in urine in an amount that negatively correlates with the life span of the organism.[10]

It is of importance that 8-hydroxy guanine, unlike guanine, can pair with both adenine and cytosine. When the repairing process does not proceed properly, the 8-hydroxy guanine and cytosine pair can be formed. Namely, a GC base pair is converted to a TA base pair through a repeated replication cycle. The daughter cell inherits the first conversion as fixed genetic information causing a mutant gene. Oxidative stress thus causes the mutation by damaging the DNA. Another protein involved in the repair mechanism for DNA mutation is MutY (Fig. 3).[11] MutY plays a role distinct

from that of 8-oxoguanine DNA glycosylase. As shown in Fig. 3, MutY recognizes and removes adenine from the 8-hydroxy guanine-adenine pair. Recently, in addition to 8-hydroxy guanine, thymine glycol has been reported to cause damage to DNA. The modified base inhibits DNA replication process and the cell is led to death.[12)]

To circumvent the oxidative stress mentioned above, the organisms have obtained a biological defense system to remove the active oxygens. The representative machinery for such a defense mechanism is the SOD system. SOD catalyzes the $2O_2^- + 2H^+ = H_2O_2$ reaction, and the resulting hydrogen peroxide is catalyzed by catalase ($H_2O_2 + AH_2 = A + 2H_2O$), or by peroxidase ($2H_2O_2 = 2H_2O + O_2$). Interestingly, SOD activity correlates positively with the life span of the organism. These results suggest that the regulation of oxidative stress could take an important therapeutic position in clinical trials.[13)]

Summary

Research on the aging mechanism in the body and the regulation of the cellular life has progressed owing to recent molecular investigations. While oxygen is indispensable to the life of the organism, it also ties in with oxidative stress. Since all of the organisms on earth evolved with this dilemma, it is important that a method of controlling oxidative stress should be developed and the effect of aging control on the organism longevity is to be further investigated.[14)]

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REFERENCES

1) Kagawa, Y.: *Bioscience on Aging.* Yodosha, Tokyo, 1996. (in Japanese)
2) Hayflick, L.: The limited *in vitro* lifetime of human diploid cell strains. *Exp Cell Res* 1965; 37: 614–636.
3) Sekiguchi, M.: *Aging and Oxidative Damage,* Iwanami Koza — Fundamental Modern Medicine, Kita, T. and Fujiwara, S. (ed.), Iwanami Shoten, Tokyo, 1999 (vol.12): 25–41. (in Japanese)
4) Kruk, P.A., Rampino, N.J. and Bohr, V.A.: DNA damage and repair in telomeres: Relation to aging. *Proc Natl Acad Sci USA* 1995; 92(1): 258–262.
5) Allsopp, R.C.: Models of initiation of replicative senescence by loss of telomeric DNA. *Exp Gerontol* 1996; 31(1-2): 235–243.
6) Collins, K.: Mammalian telomeres and telomerase. *Curr Opin Cell Biol* 2000; 12(3): 378–383.
7) Shibutani, S., Takeshita, M. and Grollman, A.P.: Insertion os specific bases during DNA synthesis past the oxidation-damaged based 8-oxodG. *Nature* 1991; 349 (6308): 431–434.
8) Dizdaroglu, M., Laval, J. and Boiteux, S.: Substrate specificity of the *Escherichia coli* endonuclease III: Excision of thymine- and cytosine-derived lesions in DNA produced by radiation-generated free radicals. *Biochemistry* 1993; 32(45): 12105–12111.
9) Tchou, J., Kasai, H., Shibutani, S. *et al.*: 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proc Natl Acad Sci USA* 1991; 88(11): 4690–4694.
10) Helbock, H.J., Beckman, K.B., Shigenaga, M.K. *et al.*: DNA oxidation matters: The HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc Natl Acad Sci USA* 1998; 95(1): 288–293.
11) Michaels, M.L., Cruz, C., Grollman, A.P. *et al.*: Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine DNA. *Proc Natl Acad Sci USA* 1992; 89(15): 7022–7025.
12) Maccubbin, A.E., Patrzyc, H.B., Ersing, N. *et al.*: Assay for reactive oxygen species-induced DNA damage: Measurement of the formamido and thymine glycol lesions. *Biochim Biophys Acta* 1999; 1454(1): 80–88.

13) Orr, W.C. and Sohal, R.S.: Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophila melanogaster. *Science* 1994; 263 (5150): 1128–1130.

14) Mecocci, P., Polidori, M.C., Troiano, L. *et al.*: Plasma antioxidants and longevity: A study on healthy centenarians. *Free Radic Biol Med* 2000; 29(8): 1243–1248.

Prehospital Management of Acute Myocardial Infarction:

—History Taking, Physical Examinations, and Laboratory Procedures—

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Abstract: Thirty percent of deaths due to acute myocardial infarction occur before patients arrive at a hospital; thus prehospital care plays a critical role in the management of this disease. Accurate history taking is the most important step in this management, as about two-thirds of all cases of acute myocardial infarction show prodromal symptoms prior to onset. Characteristic symptoms are chest discomfort, including sensations of strangulation and pressure or pain in the chest. These are often accompanied by cold sweats, nausea, vomiting, and even fear of impending death. Acute myocardial infarction should be suspected if these symptoms continue for more than 30 minutes. Physical findings may vary from slight pallor of face and an expression of agony and cold sweating in mild cases, to cyanosis or even shock in severe cases. However, it is important to recognize that these signs and symptoms are often absent. When acute myocardial infarction is suspected, an electrocardiogram should be taken immediately; blood samples should be taken to assess the white blood cell count, serum creatine kinase activity, and myocardial troponin T levels; and an echocardiogram should be obtained. Ideally, these measures should be completed within 10 minutes after a patient's arrival at a hospital.

Key words: Acute myocardial infarction; Predromal unstable angina; Silent myocardial ischemia; Infarct size; Myocardial troponin T

Introduction

It is estimated that there are 1.5 million deaths from acute myocardial infarction in the U.S. each year — i.e., one every 20 seconds.[1)] According to the Ministry of Health and Welfare, there were 31,933 recorded deaths due to acute myocardial infarction in Japan in 1990. More recently, Japan's age-adjusted mortality rate from ischemic heart disease per 100,000 population was

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determined to be approximately one-sixth of the corresponding U.S. figure.[2] Therefore, as the population of Japan is about half that of the U.S., the annual number of deaths from acute myocardial infarction in Japan could be approximately one-twelfth of the U.S. figure, or about 125,000. To reduce this high mortality, it is essential that the patients obtain early diagnosis and treatment.

Importance of Prehospital Care

The mortality rate for myocardial infarction markedly declined with the introduction of reperfusion therapies, such as thrombolysis and percutaneous transluminal coronary angioplasty (PTCA). Although the in-hospital mortality rate had been approximately 20% before reperfusion therapy, it declined to 10% or, by some accounts, to less than 5% after the introduction of reperfusion therapy. At present, therefore, the highest mortality from acute myocardial infarction occurs immediately following the onset of the disease: based on statistical figures from the 1960's, it is estimated that 30% of patients die before they arrive at a hospital. And since the mortality rate after hospital arrival is on the decline, the proportion of patients dying before arrival must be increasing. Thus, prehospital care for the treatment of acute myocardial infarction, including quick diagnosis and initiation of appropriate measures following the onset of the disease, is crucially important.

History Taking

More than half of acute myocardial infarction cases can be diagnosed through history taking. Even though there are a variety of advanced laboratory examinations available today, an accurate history is fundamental in the diagnosis of this disease.

1. Prodromal symptoms

A third of patients with acute myocardial infarction have a history of stable angina, but experience a worsening of their anginal attacks 1 to 2 weeks prior to the onset of the infarction (worsening unstable angina). Another third do not have a history of angina, but experience anginal attacks for the first time 1 to 2 weeks prior to the infarction (*de novo* unstable angina). The remaining third have no history of angina, and their first episode of angina leads to myocardial infarction. Thus, unstable angina, which is indicative of rupture of the atheroma in a coronary artery and subsequent formation of a thrombus, occurs as a prodromal symptom in two-thirds of all acute myocardial infarction cases. Infarction occurs when the thrombus is large enough to occlude the lumen of a coronary artery. Consequently, patients with acute coronary syndrome should be encouraged to enter a hospital.

2. Chest symptoms

Symptoms of acute myocardial infarction include painful feelings of strangulation or squeezing and pressure in the chest, which have been described as sensations of "suffocation," "stinging pain," "being drilled," or "burning." These symptoms occur in an area between the back of the sternum and the left anterior chest, in an area about a size of a fist. In some cases, they appear not in the precordium but in the epigastric region and are sometimes misdiagnosed as gastric ulcer. They are also radiated to the pharyngeal region and jaw in some instances. Some patients experience weakness in the left upper extremity or numbness of its ulnar side. In addition, cold sweats, nausea, and vomiting are observed in approximately half of the patients. A fear of impending death is also a characteristic of the experience.

Symptoms last for 30 minutes or longer in most cases; they may also disappear temporarily and then reappear, repeating a waxing and waning cycle. This phenomenon is presumably caused by transient interruptions in coronary blood flow, which may be due to a cycle of growth and dissolution/reduction of a throm-

Table 1 Diagnostic Criteria for Cardiogenic Shock (MIRU[4])

1. Systolic blood pressure <90 mmHg or a decrease of more than 30 mmHg from normal
2. Presence of circulatory failure in internal organs caused by low cardiac output
a. Hourly urinary output <20 m*l*
b. Disturbed consciousness
c. Peripheral vasoconstriction with cold, wet skin
Exceptions: vasovagal reflex, arrhythmias, and low blood pressure due to hypovolemia following drug administration.

MIRU: Myocardial Infarction Research Unit

Table 2 Blood Pressure at the Time of Hospital Arrival and In-hospital Mortality Rates

Systolic blood pressure (mmHg)	Number of cases	In-hospital mortality rates
~90	65 cases (9.7%)	26.2%
91~100	50 cases (7.5%)	22.0%
101~120	149 cases (22.3%)	14.8%
121~140	189 cases (28.3%)	11.1%
141~	215 cases (32.2%)	5.6%

Kinki University CCU (1982–1991)

bus in a coronary artery, or to the appearance and disappearance of coronary spasms. Symptoms gradually diminish a few hours after onset and disappear within approximately half a day, leaving a mild sensation of pressure in the chest. In some cases, the symptoms completely disappear early after onset, which is indicative of dissolution of the occluding thrombus and the reestablishment of coronary blood flow (spontaneous recanalization).[3]

3. Silent myocardial infarction

Twenty to sixty percent of myocardial infarctions are detected by chance on an electrocardiogram taken at a periodic medical check-up, or they may be discovered at autopsy. In half of these cases, information about symptoms pointing to the possible presence of myocardial infarction can be obtained by history taking. On the other hand, no such symptoms are observed in the remaining half of these cases. Those with a history of angina, hypertension, or diabetes are more likely to have such silent myocardial infarctions.

4. Other symptoms

Fainting may occur due to bradycardia caused by complete atrioventricular block, which is most frequently observed with inferior infarctions. Fainting can also be caused by malignant ventricular arrhythmias (ventricular fibrillation or ventricular tachycardia). Indeed, ventricular fibrillation accounts for a large fraction of the deaths from acute myocardial infarction occurring within 30 minutes of disease onset. Cardiogenic shock may occur when the size of the infarct is large and there is an episode of left ventricular failure, leading to a decrease in blood pressure (Table 1). If so, peripheral circulatory dysfunction and disturbed consciousness are observed; breathing difficulty and cyanosis are noted in cases where the left ventricular failure results in pulmonary congestion.

Physical Findings

1. General condition

Although no physical abnormalities are observed in mild cases, the patients often look pale and show an expression of anguish. Patients with shock also experience cold sweats, decreased skin temperature, cyanosis, and disturbed consciousness. Body temperature does not rise on the first day after disease onset, but gradually increases thereafter. If a rise in temperature is noticed immediately following disease onset, other disorders, such as acute pericarditis, should be suspected. Tachycardia is frequently observed due to increased sympathetic nerve activity. Some patients, however, may present with sinus bradycardia due to increased vagal tone, or bradycardia resulting from atrioventricular block. Elevated blood pressure due to increased sympathetic nerve activ-

Table 3 Killip Classification and In-hospital Prognosis

Killip classification	Number of cases*	Number of deaths*
Class I: No clinical evidence of heart failure	487 cases	40 cases (8.2%)
Class II: Mild to moderate heart failure (Rales in up to 50% of all lung fields)	76 cases	20 cases (26.3%)
Class III: Pulmonary edema (Rales in more than 50% of all lung fields)	29 cases	14 cases (48.3%)
Class IV: Cardiogenic shock (Accompanied by blood pressure<90 mmHg, diminished urinary output, cyanosis, cold and wet skin, and disturbed consciousness)	43 cases	23 cases (53.5%)
	635 cases	97 cases (15.3%)

*Number of cases and deaths Source: Kinki University CCU (1982–1991)

ity is also seen in mild cases. Because decreased blood pressure is observed primarily in severe cases, hypotension at the first medical examination suggests a poor prognosis (Table 2).

2. Pulmonary auscultation

Bubbling rales are heard in patients with pulmonary congestion. Careful auscultation should be performed posteriorly to both the left and right lower lung fields while the patient sits still. The Killip classification (Table 3), which is based on the magnitude of the rales heard, is important for classification of the severity of a myocardial infarction.

3. Cardiac auscultation

The first sound is attenuated on the first day after the onset of acute myocardial infarction. In patients with left ventricular diastolic dysfunction, the third sound is often audible. Papillary muscle dysfunction is suspected when a loud systolic murmur is heard in the area between the left sternal edge in the 4th intercostal space and the apex. Patients with papillary muscle rupture or perforation of the interventricular septum experience thrills. These can be readily diagnosed using echocardiography or Doppler examination. Pericardial friction rubs are heard in 6% to 30% of patients with acute myocardial infarction; detection requires careful auscultation, however, as they are transient and localized.

Table 4 Useful Tests for Prehospital Diagnosis of Acute Myocardial Infarction

Electrocardiography
Echocardiogram
Blood myocardial Troponin T levels
Leukocyte counts
Serum creatine kinase activity

4. Other findings

Palpation should always be performed on the left and right dorsal pedis arteries of patients with myocardial infarction. The inability to locate these arteries indicates the presence of arteriosclerosis obliterans, which is one of the important factors influencing patient prognosis.

Laboratory Examinations

When acute myocardial infarction is suspected, the tests listed in Table 4 should be performed immediately.

(1) **Electrocardiogram (ECG)**: ECG is the most important examination tool. For that reason, one should strive continuously to optimize one's ability to use ECG for diagnosing acute myocardial infarction.

(2) **Echocardiography**: Echocardiography is the second most useful examination tool. When abnormal left ventricular wall motion detected by echocardiography matches the extent of the ST segment elevation on an ECG, myocardial infarction is almost certain.
(3) **Leukocytes**: The white blood cell count is an important indicator because it increases within 2 hours after disease onset. One drawback, however, is that it can also increase in diseases other than myocardial infarction.
(4) **Serum creatine kinase activity (CK)**: Elevation of serum CK levels is seen 3 to 4 hours after disease onset, immediately following the increase in the leukocyte count. As with white blood cell counts, determination of serum CK levels takes time because blood samples must be sent to the laboratory for analysis.
(5) **Myocardial troponin T**: Blood levels of myocardial troponin T can be readily detected using a commercially available testing kit. This enables a diagnosis of myocardial infarction to be made within 15 minutes, merely by applying a drop of blood to test paper at the bedside.

Differential Diagnosis

Differential diagnosis is sometimes necessary to distinguish chest symptoms caused by acute myocardial infarction from those caused by hypertension, valvular diseases, myocardial diseases, aortic dissection, pulmonary embolism, pneumothorax, gastrointestinal disease, intercostal neuralgia or herpes zoster. Differential diagnosis is also necessary in cases of cardiac neuralgia or chest pain syndrome. In most cases, acute myocardial infarction can be differentiated by careful analysis of the symptoms with the aid of an ECG and a troponin T test.

Conclusion

If you see a patient who complains of chest symptoms, acute myocardial infarction should be suspected, and in-depth questioning should be conducted. When you suspect the disease, ECG should be performed immediately, after which blood tests for myocardial troponin T, white blood cell counts, and myocardial CK activity should be performed. Echocardiography should then be carried out while waiting for the results of the blood tests. If the diagnosis is confirmed, treatment measures, such as intravenous thrombolysis, may be indicated. When acute myocardial infarction is suspected, or the diagnosis cannot be confirmed, the patient should be transferred to a hospital with comprehensive disease management capabilities.

REFERENCES

1) Antman, E.M. and Braunwald, E.: Chapter 24 Acute myocardial infarction. *In:* Braunwald, E., edit: Heart Disease. Philadelphia, Saunders, 1997 5th edition. pp. 1184–1365.
2) Tunstall-Pedoe, H., Kuulasmaa, K., Amouyel, P., Arveiler, D., Rajakangas, A-M. and Pajak, A.: Myocardial infarction and coronary deaths in the World Health Organization MONICA project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. WHO MONICA project. *Circulation* 1994; 90: 583–612.
3) Akiyama, H., Ishikawa, K., Kanamasa, K., Ogawa, I., Koka, H., Kamata, N., Nakai, S. and Katori, R.: Increased coronary vasomotor tone in acute myocardial infarction patients with spontaneous coronary recanalization. *Jpn Circ J* 1997; 61: 503–509.
4) Swan, H.J.C., Forrester, J.S., Danzig, R. and Allen, H.N,: Power failure in acute myocardial infarction. *Prog Cardiovasc Dis* 1970; 12: 568–600.

Diagnostic Approach in the Elderly

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Abstract: At the onset of acute myocardial infarction (AMI) in the elderly, atypical manifestations, such as symptoms of heart failure, gastrointestinal symptoms and disturbance of consciousness, increase with aging. Moreover, early diagnosis is important so as not to miss the optimal period for coronary artery reperfusion therapy. Because elderly patients tend to develop complications, and their mortality rate is high, the assessment of dementia, activities of daily living and complications involving other organs (especially cerebrovascular disease, renal dysfunction and aortic aneurysm) in addition to taking the history and physical findings is important in deciding the indication of reperfusion therapy. Since many elderly patients often have ECG abnormalities, such as bundle branch block and ST,T changes, if prior ECGs are available, the present ECG should be compared with prior ECGs. Because of lack of the elevation in CPK values often within 3 hours after the onset of AMI, the measurement of troponin-T is useful as a means of improving specificity and prompt diagnosis for myocardial ischemia. Echocardiography is useful in the diagnosis of high posterior wall, lateral wall and non-Q wave myocardial infarction, which are difficult to diagnose electrocardiographically. Thus, comprehensive examinations including ECG, serum enzymes and echocardiography are necessary, irrespective of the clinical symptoms in the elderly.

Key words: Elderly; Painless myocardial infarction; Reperfusion therapy; Complications in other organs

Introduction

Acute myocardial infarction (AMI) in the elderly is characterized in comparison with the middle aged patient as follows, 1) more cases in women, with the lack of sex difference, 2) more painless or asymptomatic cases, 3) old and non-Q wave infarctions are common due to a high proportion of multivessel coronary artery disease, 4) heart failure, shock and cardiac rupture are relatively common, and prognosis is poor, 5) a high rate of complications involving other organs with aging, such as cerebrovascular disease and renal dysfunction.

Thus, despite the presence of serious underlying conditions, the clinical manifestations are

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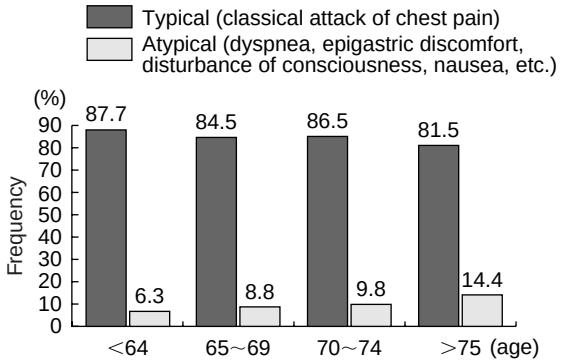

Fig. 1 Symptoms during the onset of myocardial infarction (cited from literature reference No.1)

Table 1 Predominant clinical symptoms and circumstances of acute myocardial infarction in the aged ($n = 153$)

Chest pain	56	36
Dyspnea	29	19
Shock	26	17
Consciousness disturbance	20	13
Infection	7	5
Operation	5	3
Anorexia	4	3
Weakness	3	2
Unknown	3	2
Total	153 cases	100%

mild in the elderly. Elderly patients tend to develop complications and the mortality rate tends to increase. Therefore, early diagnosis of AMI is more important so as not to miss the optimal period for coronary artery reperfusion therapy.

Manifestations of AMI in the Elderly

The typical symptom is chest pain, characterized by a squeezing sensation and a feeling of pressure in the center of the sternum, that persists for a long time and is so severe that nitrates are ineffective and narcotics are required. However, a typical chest pain decreases with aging. Atypical manifestations, such as symptoms of heart failure (dyspnea, shock), gastrointestinal symptoms (vomiting, upper abdominal pain) and disturbance of consciousness, tend to increase with aging even among patients admitted to the coronary care unit (CCU) within the first 24 hours after the onset of AMI[1] (Fig. 1).

In our study of autopsy cases with AMI, the incidence of chest pain was only 36%. Cases occurring under such circumstances as infection or postoperative states and cases with only nonspecific symptoms, such as anorexia or weakness, were found[2] (Table 1). Cerebrovascular disease, in particular, impairs activities of daily living (ADL) and communication. These disorders are largely responsible for asymptomatic myocardial ischemia.[3] In addition, simultaneous occurrence of AMI and stroke is not rare. Thus, comprehensive examinations including ECG, serum enzymes and echocardiography are necessary, irrespective of the clinical symptoms in the elderly.

Diagnosis of AMI in the Elderly and Points Requiring Caution

(1) Taking history and physical examination: It is important to take history carefully that includes coronary risk factors and to identify the time of onset. However, taking history in the elderly is often difficult by reason of uncertain memory, dementia and living alone.

The time from the onset of AMI is important when deciding on the indication of coronary artery reperfusion therapy (usually within 6 to 12 hours). Since the survival rate is higher if reperfusion therapy can be performed in the early stage, especially in the elderly,[4] it is important to estimate the time from the onset of AMI taking adequate history that includes information from the family. The assessment of dementia, activities of daily living and complications involving other organs (especially cerebrovascular disease, renal dysfunction and aortic aneurysm), in addition to taking the history and physical findings, is important when deciding on the indication of reperfusion therapy.

(2) ECG: Many elderly patients often have ECG abnormalities, such as bundle branch

block and ST,T changes, and also exhibit non-Q wave infarction with only ST depression or negative T wave. In cases with left bundle branch block or right ventricular pacing wave it is difficult to diagnose AMI from the ECG findings. Therefore, the ECG should be made out with caution, and if prior ECGs are available, the present ECG should be compared with them. In addition, AMI-like ECG changes may be caused by disseminated intravascular coagulation (DIC), blood transfusion, pneumonia, infection and cerebral infarction in the elderly.

(3) Serum enzymes: Elevation of creatine phosphokinase (CPK) value is not often found within 3 hours of the onset of AMI. In regard to measurement of the CPK value, there are some problems as follows: 1) myocardial specificity (particularly differentiation from the increase due to circulatory failure in patients with heart failure), 2) prompt diagnosis for myocardial ischemia, 3) short duration of abnormal values. A useful kit for measuring troponin-T (TROP-T®), which enables the quantitative measurement of a small sample of whole blood, has recently become commercially available as a means of improving specificity and prompt diagnosis for myocardial ischemia. In addition, another kit for measuring myocardial fatty acid binding protein (H-FABP) as an early marker of myocardial ischemia has been developed.

(4) Echocardiography: If abnormal motion in the left ventricular wall that corresponds to an ST,T change in the ECG is shown in the echocardiogram, the diagnosis of myocardial ischemia or infarction is definitive. Echocardiography is useful for the diagnosis of high posterior wall, lateral wall and non-Q wave infarctions, which are difficult to diagnose electrocardiographically. However, some elderly patients show poor visualization of the left ventricular short axis view because of pulmonary emphysema or an S-shaped interventricular septum. In those cases, the diagnosis of myocardial ischemia is difficult in the territory of the left circumflex and right coronary artery.

Conclusions

The procedure for early diagnosis of myocardial ischemia and to transfer elderly patients from the primary care clinic to CCU is as follows; (1) Take a precise history and physical examination, and always record the ECG regardless of whether cardiac symptoms are present. (2) Since many elderly patients often have ECG abnormalities, if prior ECGs are available the present ECG should be compared with them. (3) In addition to the routine blood examination, if possible, determine whether myocardial ischemia is present by using a prompt diagnosis kit, such as TROP-T®. (4) If myocardial ischemia or infarction is diagnosed in the acute phase after the onset of AMI, transfer the patients to the nearest CCU with their medical history and ECGs.

REFERENCES

1) Hosoda, S.: Study on treatment of myocardial infarction in the aged. In: *Advances in Aging and Health Research 1994*. Japan Foundation For Aging and Health, 1994, pp.61–71. (in Japanese)
2) Oda, S., Matsushita, S., Toda, G. *et al.*: The painless myocardial infarction in the aged. *Japanese Journal of Geriatrics* 1986; 23: 600–604. (in Japanese)
3) Nakahara, K., Matsushita, S., Yamanouchi, H. *et al.*: Relation of asymptomatic myocardial ischemia to impaired communication, cerebrovascular disease, and lack of ability to perform activities of daily living in elderly patients. *Japanese Journal of Geriatrics* 1997; 34: 285–291. (in Japanese)
4) The Japanese Acute Myocardial Infarction in the Elderly Study Group: Guideline for treatment of acute myocardial infarction in the elderly. *The Journal of the Japanese Society of Internal Medicine* 2000; 89: 175–182. (in Japanese)

Report on the Criteria for the Determination of Brain Death in Children

—1999 Report of the Study Group on the Criteria for Determination of Brain Death in Children, Ministry of Health and Welfare—

Part I: Survey of Clinical Practices in the Diagnosis of Brain Death in Children in Japan

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Key words: Brain Death; Children; Epidemiology

Introduction

In Japan, there has been increasing demand for the establishment of criteria for the diagnosis of brain death in young children, since the criteria proposed by the Brain Death Study Group of the Ministry of Health and Welfare (MHW) (Table 1) in 1985[1,2] excluded children under 6 years of age. This prompted us to conduct a national survey of the clinical practice on brain-dead or near-brain-dead children (a state strongly suggestive of brain death), that we expected may allow us to develop criteria for the diagnosis of brain death in children.

Methods and Subjects

The subjects of the survey included neonates, premature infants, infants and young children, who were diagnosed to be either brain-dead or near-brain-dead, regardless of whether the cause of the brain damage was primary or secondary. A total of 1,220 institutions that were considered likely to encounter pediatric brain death were registered for the survey.

Reports on all the cases, even if the forms were partially unfilled with respect to some items of the protocol, were requested. The form consisted of the following items: clinical diagnosis at presentation, clinical course, cause of brain death, estimated interval between the causal event and the diagnosis of brain death, clinical parameters and laboratory tests (vital signs, use of drugs, mode of mechanical ventilation, apnea test, level of consciousness, brainstem reflexes (light reflex, corneal reflex, ciliospinal reflex, oculocephalic reflex, vestibular reflex, pharyngeal reflex, and cough reflex), pupillary diameter, spinal reflex, neurophysi-

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Table 1 Criteria Proposed by the Ministry of Health and Welfare (Takeuchi's Guidelines)

Subjects
- Patients who have been in a deep coma and are apneic as a result of organic brain damage.
  *"Apnea" here refers to the absence of spontaneous respiration during mechanical ventilation or no resistance to mechanical ventilation. No vigorous apnea test is necessary.
- Patients in whom the cause has been definitively diagnosed and in whom the nature of the damage has been judged to be irremediable, despite all currently available treatment methods for the disease.

Exclusion criteria
- Children (under 6 years of age)
- Acute drug poisoning
- Hypothermia (32°C or lower core temperature)
- Metabolic or endocrine disorder

Caution before judgment
- Exclude the effects of drugs acting on the central nervous system (the dose, dosing method, duration, time after the last dose, and the effective activity period of the drug should all be taken into consideration) and muscle relaxants (nerve stimulation should be performed). The systolic blood pressure should be 90 mmHg or more.

Diagnostic criteria (Bold letters denote the three main elements)
- **Deep coma** (a score of 300 on the Japan Coma Scale)
- **Pupillary diameter** (4 mm or more on both sides), fixed
- **Absence of brainstem reflexes**
  Light, corneal, ciliospinal, oculocephalic, vestibular, pharyngeal, and cough reflexes (Test separately on each side).
- Flat EEG (A sensitivity of 2.5μV/mm or more is necessary, according to the guidelines of the Japanese Society of Electroencephalography and Electromyography.)
- Absence of spontaneous respiration ($PaCO_2 \geqq 60$ mmHg during suspension of mechanical ventilation in the apnea test. Although the method for suspending mechanical ventilation is arbitrary, the administration of 100% oxygen before and during the test is essential.)
  *The apnea test should be the last in the test series.

Observation period
- Confirmation of the absence of neurological changes after 6 hours (This does not mean that the second examination should be performed exactly 6 hours later).
- A period of more than 6 hours should be allowed for patients with secondary brain damage (e.g., after cardiopulmonary resuscitation), and in children 6 years old or older.

Sources: Report of the Study Group on Brain Death: Guidelines and criteria for diagnosis of brain death. *The Journal of the Japan Medical Association* 1985; 94: 1949–1972.
Supplement for brain death criteria by the Brain Death Study Group of the Ministry of Health and Welfare. *The Journal of the Japan Medical Association* 1991; 105: 525–546.

ological tests, diagnostic imaging of the brain, cerebral blood flow test and autopsy. The period of the survey was from April 1, 1987 to April 30, 1999. A set of provisional criteria (Table 2) for diagnosing pediatric brain death had been sent to each institution in May, 1998, so as to provide some tentative guidelines. A prospective and retrospective survey was conducted before and after provision of the provisional criteria, respectively.

The provisional set of criteria was essentially similar to the criteria proposed by the MHW, but was modified in relation to the exclusion cases and the interval for reassessment, based on previously reported data and the criteria laid down in various other countries.

Results

(1) Recovery of case report forms

A total of 162 case report forms were returned. Responses were obtained from 67 of the 1,220 institutions, with a response rate of 5.5%. About half of all the responses were from pediatric departments.

Twenty-three cases were excluded from the

Table 2 Criteria for Diagnosing Brain Death in Children (Provisional Criteria)

Premise

Brain death is a clinical diagnosis judged to be irreversible, with total loss of brain function as a result of organic brain damage.

The present criteria are provisional criteria for the clinical diagnosis of brain death in children under 6 years of age.

Subjects and exclusion criteria

[Subjects]

The present criteria are applicable to children under 6 years of age.

The patients should be in a deep apneic coma as a result of organic brain damage; the cause of brain death should be definitively diagnosed, and the nature of the damage should have been judged to be irremediable despite maximal attempts at treatment.

Therefore, the following cases should be excluded.

[Exclusions]

1. Cases in which the cause of the deep coma or the absence of spontaneous respiration is unknown at the time of assessment.
2. Cases in whom there is a possibility that the cause of the deep coma and the absence of spontaneous respiration may be reversible at the time of assessment.

Hypothermia: Body temperature of 32°C or less

Hypotension: Markedly low blood pressure not appropriate for age

Marked hypoxia

Cases in whom there is a strong possibility that the deep coma and absence of spontaneous respiration are attributable to muscle relaxants or central nervous system depressants.

Cases in whom the deep coma and absence of spontaneous respiration are attributable to metabolic or endocrine disorders.

Diagnostic criteria

1. Deep coma
2. Moderate or more dilation and fixation of the pupils, bilaterally
3. Absence of brainstem reflexes
4. Flat EEG
5. Absence of spontaneous respiration

The above five items should be assessed and reassessed at the following intervals, according to the patient's age. However, confirmation of the absence of spontaneous respiration should be performed at the end of each assessment.

Fixation of the pupils implies the absence of a light reflex.

[Interval for reassessment (observation period)]

Assessments of all five items should be repeated twice at the following intervals.

Neonates younger than 28 days	48 hours
Infants younger than 1 year	24 hours
Children 1 year old or older, but younger than 6 years	12 hours

Doctors in charge of assessment

Two or more doctors who are not involved in organ transplantation should assess the patient. At least one doctor should be a specialist who has experience in dealing with brain death in children. The other doctor could be the doctor who treated the patient. (The term "specialist" refers to a neurosurgeon, pediatric neurologist, emergency specialist, or anesthesiology/resuscitation/intensive care specialist who has received accreditation from their respective medical societies.)

Adjunctive examinations

Brainstem auditory evoked potential examination, cerebral angiography, RI brain scintigraphy (SPECT, etc.), Doppler ultrasound examination, and Xe-CT provide useful information, but these examinations are not essential.

analysis, as they were considered to be unsuitable for inclusion in the present survey of brain-dead or near-brain-dead children under 6 years of age. In all of the 162 cases, cardiac arrest eventually occurred after the diagnosis of brain death.

(2) Classification of cases

The cases included in the study were classified into 4 groups, as shown in Table 3, based on the number of apnea test and neurological testing sessions.

Since the apnea test is of great importance in

Table 3 Classification of Cases

	Definition		No. of cases (%)
	Apnea test	Neurological testing	
Group I	At least 2 sessions	At least 2 sessions	20 (14%)
Group II	1 session	At least 1 session	10 (7%)
Group III	None	At least 2 sessions	65 (47%)
Group IV	None	1 session	44 (32%)

the diagnosis of brain death, cases for which strictly 2 or more sessions of the apnea test were conducted were classified into Group I. This group represents that in which the diagnosis of brain death was made in greatest compliance with the provisional criteria, and comprised 20 cases (including 11 cases in the prospective survey). Cases for which at least one session of neurological testing was conducted, but in whom the apnea test was performed only once, were classified into Group II. All the 10 cases in this group were those included in the retrospective survey.

Cases in whom brain death was diagnosed without the apnea test were classified into Group III (2 or more sessions of neurological testing) or Group IV (only one session of neurological testing). Considering the fact that the apnea test was not performed in these cases, it is impossible, in a strict sense, to be sure that these cases were brain-dead. However, all of these cases were judged by the doctor-in-charge to be brain-dead or near-brain-dead. All these patients were in deep coma, required mechanical ventilation, and eventually developed cardiac arrest. Therefore, the cause of the brain damage, the neurological findings, and the information obtained from diagnostic imaging procedures in these cases also were considered to be useful for the development of criteria for the diagnosis of brain death in the pediatric age group.

Responses were either simply summed up as a whole, or according to the classification of the cases for each item, including "unclear" items. For some items, cross summation was used to test the relationships among the items and among the groups. For the cross summation, the chi-squire test was used, with the significance level set at 5%.

(3) Case characteristics

1) Sex and age

The 139 cases included for the analysis comprised 83 males and 56 females, including 5 neonates less than 28 days old (one case each 5, 6, 14, 15 and 25 days old), and 9 infants less than 12 weeks old. One-year-old infants, 39 in all, accounted for the largest percentage of the population. There were 108 children who were between 5 months and 4 years of age, accounting for 78% of all the cases (Fig. 1).

2) Cause of brain damage

Primary brain damage (head trauma, encephalitis, encephalopathy, intracranial bleeding, etc.) accounted for 79 cases (Fig. 2), and secondary brain damage (suffocation, drowning, hypoxia, etc.), for 60 cases. Head trauma was the most frequent cause of primary brain damage. In regard to sex, head trauma and drowning were more frequent among boys. Accidents, such as suffocation and drowning, were prominent among the causes of secondary brain damage. Cases reported as sudden infant death syndrome (SIDS) were classified under the category of cardiopulmonary arrest. Such conditions are, in a strict sense, referred to as apparently life threatening events (ALTE) in infants.[3,4]

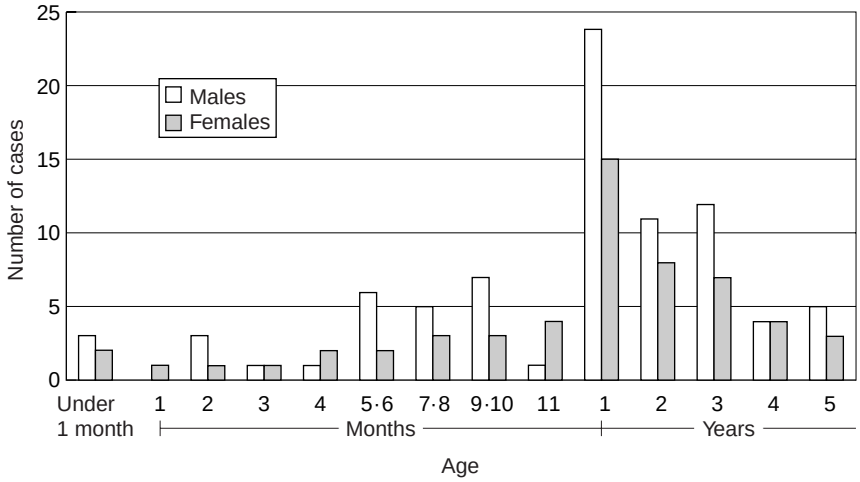

Fig. 1 Age and sex distributions

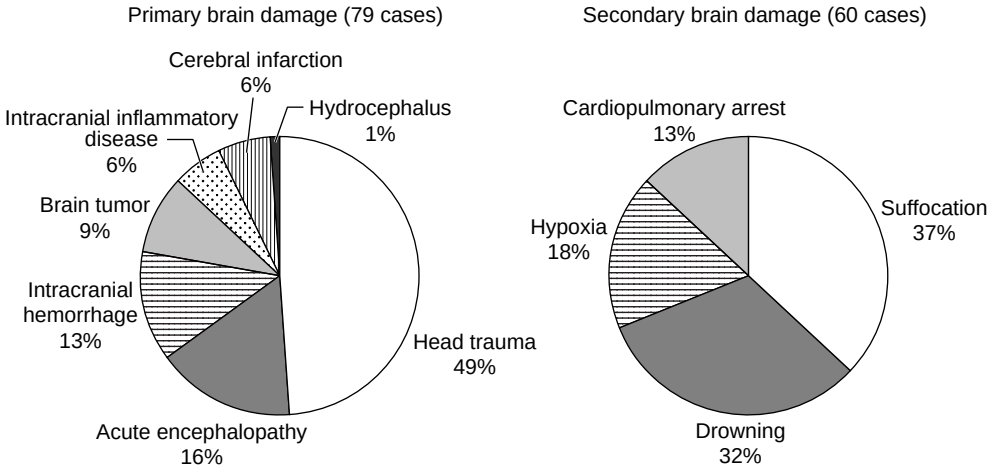

Fig. 2 Causes of brain death

The ratio of the primary to secondary causes of brain damage in children was characteristically different from that in adults. According to the MHW study,[1] primary brain damage was overwhelmingly more frequent in adults than secondary brain damage, with a primary : secondary ratio of 92 : 8. The corresponding ratio in children in the present survey was 57 : 43.

(4) Clinical findings

1) Vital signs

Changes in body temperature, pulse rate, blood pressure, etc. are not necessarily specific for the diagnosis of brain death, because a variety of factors affect these parameters.

2) Consciousness

All the cases were rated as having a score of 300 on the Japan Coma Scale (JCS),[5] or 3 on the Glasgow Coma Scale (GCS).[6,7]

3) Pupillary diameter

In Groups I and II, the pupillary diameter on both sides was 4 mm or greater, regardless of

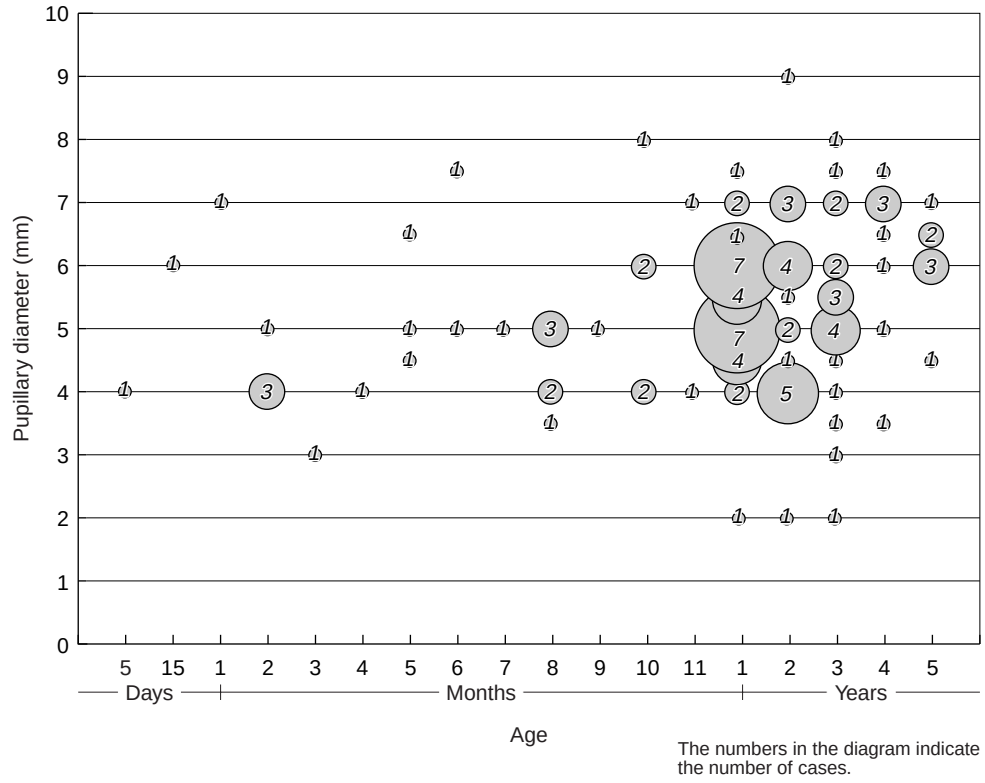

Fig. 3 Age and pupillary diameter (right)

the patients' age, with a predominance of cases having a pupillary diameter in the range of 5.0–5.9 mm (Fig. 3). In Groups III and IV, the pupillary diameter was less than 4 mm on the left side in 7 cases and the right side in 8 cases, accounting for a total of about 7% of the cases.

4) Brainstem reflexes

As shown in Table 4, in Groups I and II, all the brainstem reflexes were absent in all of the cases, excluding three in which data on the vestibular reflex were lacking. In Groups III and IV, the light reflex was the most frequently tested among the brainstem reflexes, followed by the cough, corneal, pharyngeal, oculocephalic, and ciliospinal reflex, in that order; the vestibular reflex was the least frequently tested. In Group III and IV, the light reflex was absent in all the cases for which data were available. However, for the other brainstem reflexes, the data were missing for a large number of cases; in particular, absence of the vestibular reflex was confirmed in only a small number of cases. In general, no consideration was given to vestibular reflex testing in cases where the apnea test was not considered.

5) Respiration — Apnea test

The apnea test was positive (i.e., the patient was apneic) in all of the tested cases in whom arterial blood gas analysis was performed. At the first examination in Group I, the mean $PaCO_2$ and PaO_2 were 41 ± 6 and 376 ± 153 mmHg before the test, and 81 ± 13 and 332 ± 165 mmHg at the end of the test, respectively, and the mean systolic blood pressure was 98 ± 21 mmHg during the test. At the second examination, the mean $PaCO_2$ and PaO_2 were 42 ± 8 and 369 ± 129 mmHg before the test, and 82 ± 15 and 342 ± 127 mmHg at the end of the test,

Table 4 Brainstem Reflexes

No. of cases (%)

Group	I		II		III		IV	
Brainstem reflexes	Absence	Unknown	Absence	Unknown	Absence	Unknown	Absence	Unknown
Light reflex	20 (100)	0	10 (100)	0	64 (98)	1	42 (95)	2
Corneal reflex	20 (100)	0	10 (100)	0	44 (68)	21	26 (59)	18
Ciliospinal reflex	20 (100)	0	10 (100)	0	30 (46)	35	16 (36)	28
Oculocephalic reflex	20 (100)	0	10 (100)	0	36 (55)	29	16 (36)	28
Vestibular reflex	19 (95)	1	8 (80)	2	9 (14)	56	2 (5)	42
Pharyngeal reflex	20 (100)	0	10 (100)	0	41 (63)	24	21 (48)	23
Cough reflex	20 (100)	0	10 (100)	0	50 (77)	15	29 (66)	15

respectively, and the mean systolic blood pressure during the test was 90 ± 18 mmHg. In Group II, the mean $PaCO_2$ and PaO_2 were 40 ± 10 and 297 ± 161 mmHg before the test, 72 ± 22 and 187 ± 162 mmHg at the end of the test, respectively, and the mean systolic blood pressure during the test was 101 ± 34 mmHg.

The provisional set of criteria tentatively prescribes a $PaCO_2$ value of 60 mmHg or higher as the cutoff level. The results of the present study indicate that the mean $PaCO_2$ at the end of the test was about 80 mmHg. No particular problems during the apnea test were recorded. The $PaCO_2$ and systolic blood pressure during the test also indicated appropriate maintenance of oxygenation and blood pressure.[8–11)]

6) Spinal reflex

The spinal reflex[12,13)] was positive in 8 of the 30 cases in Groups I and II, and negative in 12; data were not entered for 10 cases. In Groups III and IV, the spinal reflex was positive in 12 of the 109 cases, and the data were not entered for 57 cases.

(5) Neuro-imaging and laboratory examinations

1) Neuro-imaging (Table 5)

Cranial CT was the most frequently (90%) performed imaging procedure in Groups I and II (Table 5). Even in Group IV, the percentage of patients in whom the test was conducted was 82% (Table 5).

2) EEG and brainstem auditory evoked potentials

EEG was performed in all cases in Groups I and II. This examination was also performed in 83% of cases in Group III and 57 % of cases in Group IV. So-called flat EEG was found in all of the cases examined.

Brainstem auditory evoked potential testing was performed in 95%, 60%, 51% and 43% of cases in Groups I, II, III and IV, respectively. In Groups I and II, all the brain waves after the II wave were lost in all of the 25 cases examined, but persistent I waves were observed in 2 cases. In Groups III and IV, of 38 cases for which the data on I waves were available, all

Table 5 Diagnostic Imaging Examinations

No. of cases (%)

Method \ Group	I		II		III		IV	
	Used	Not used	Used	Not used	Used	Not used	Used	Not used
Cranial CT	18 (90)	2	9 (90)	1	54 (83)	11	36 (82)	8
Cranial MRI	5 (25)	15	0 (—)	10	3 (5)	62	1 (2)	43
Cranial ultrasonography	3 (15)	17	0 (—)	10	5 (8)	60	1 (2)	43

Table 6 Cerebral Blood Flow Examination

No. of cases (%)

Method \ Group	I		II		III		IV	
	Used	Not used	Used	Not used	Used	Not used	Used	Not used
Transcranial Doppler	9 (45)	11	2 (20)	8	7 (11)	58	2 (5)	42
RI cerebral blood flow	6 (30)	14	4 (40)	6	8 (12)	57	2 (5)	42
Angiography	3 (15)	17	1 (10)	9	3 (5)	62	2 (5)	42

the brain waves were lost in 28, whereas persistent I waves were found in 10 cases.

3) Cerebral circulation

Transcranial Doppler flowmetry and RI cerebral angiography were performed in some of the patients (Table 6). In Groups I and II, transcranial Doppler flowmetry was performed in 37% of the cases. In contrast, cerebral angiography was performed in fewer than 15 % of the cases.

4) Various examinations in relation to age

Among the 139 cases, differences in the age distribution between the group of cases examined by the various tests (EEG, cranial CT, cranial MRI, cranial ultrasonography, transcranial Doppler flowmetry, RI cerebral angiography) and the group not examined by these tests were analyzed by the chi-squire test. Cranial ultrasonography was significantly more frequently performed in younger than older children. This is probably because the anterior fontanelle is more often open in younger children. There were no significant differences in the age distribution in relation to the other examinations.

(6) Use of drugs

Vasopressors were used in 68–83% of cases in each of the groups. Anticonvulsants and sedatives were used in 10–22% of cases.

(7) Interval to reassessment

In cases in which 2 or more assessments were carried out, the interval between the first and the second assessment was analyzed. Influence of the provisional criteria was considered possible in the prospective survey. Therefore, such

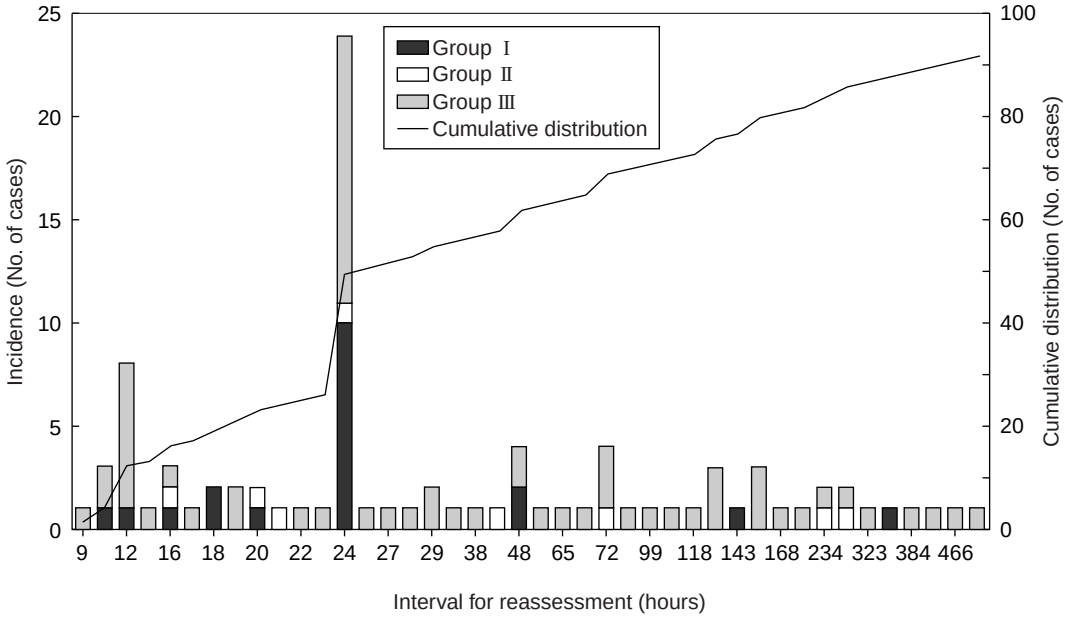

Fig. 4 Interval for reassessment in 92 cases

cases were taken into consideration in this analysis. A total of 92 cases were included in the analysis of the interval between repeat assessments. These cases included all of the 20 cases from Group I, 8 cases from Group II in the retrospective survey, and 64 cases from Group III, excluding 1 case for which the relevant data were not clearly recorded (55 in the retrospective survey and 9 in the prospective survey).

1) Interval to reassessment — observation period

Among the 92 cases, the interval to reassessment was under 12 hours in 12 cases, under 24 hours in 50 cases, and under 48 hours in 62 cases (cumulative rate, 67%), as shown in Fig. 4. In these 62 cases, the peak interval was found to be 24 hours (24 cases), followed by a second peak at 12 hours (8 cases). This indicates that many physicians regard 24 hours as the standard interval for reassessment.

2) Relation to age

When the interval between two assessments was analyzed according to the age of the children, namely, when the children were grouped into neonates, infants and young children, there were two neonates in whom the diagnostic process was repeated after 48 hours and 234 hours, respectively. Among infants, the diagnostic process was repeated within 12 hours in 2 cases, within 24 hours in 13 cases, and within 48 hours in 18 cases (60%), out of the total of 30 cases. In contrast, in young children, the reassessment was carried out within 12 hours in 10 cases, within 24 hours in 37 cases (62%), and within 48 hours in 43 cases (72%), out of the total of 60 cases. Thus, the interval tended to be longer in the group of young children, as compared to that in the case of infants and neonates (Fig. 5). This finding suggests that the standard interval for reassessment may be 24 hours in infants, and 12 hours or 24 hours in young children.

3) Relationship to the cause of brain death

In cases with primary brain damage, the interval to reassessment was under 24 hours in about 70%, whereas in cases with secondary brain damage, the interval was under 48 hours

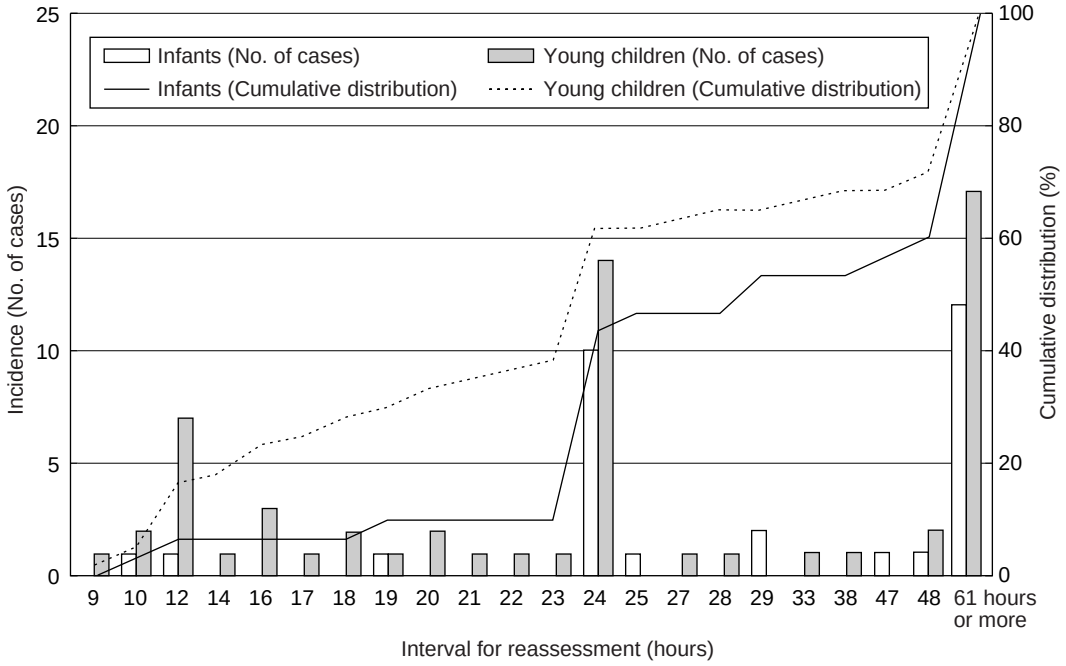

Fig. 5 Age and interval for reassessment

in about 60%. Thus, there was a tendency for cases with secondary brain damage to be reassessed after longer intervals. With regard to the age structure, primary and secondary brain damage accounted for 1 case each among neonates, 12 cases (24%) and 18 cases (42%), respectively, among infants, and 36 cases (73%) and 24 cases (56%), respectively, among young children. These differences in the age structure may be responsible for the tendency towards longer intervals to reassessment in cases with secondary brain damage.

There was no significant difference in the interval to reassessment between cases infants with primary and secondary brain damage. On the other hand, analysis of the interval to reassessment in young children with primary and secondary brain damage revealed that among young children, the interval was under 24 hours in about 70% of children with primary brain damage, and 48 hours in 54% of children with secondary brain damage, reflecting a tendency towards longer intervals in children with secondary brain damage. Overall, the maximum interval was 24 hours in both cases of primary and secondary brain damage.

4) Relation to the type of survey

Among the 20 cases in the prospective survey, the interval to reassessment was under 12 hours in 4 cases (cumulative rate, 20%), under 24 hours in 14 cases (70%), and under 48 hours in 17 cases (85%). Among the 72 cases in the retrospective survey, the interval was under 12 hours in 8 cases (cumulative rate, 11%), under 24 hours in 36 cases (50%), and under 48 hours in 45 cases (63%). The interval was thus shorter in the cases in the prospective survey, i.e., those cases in whom brain death was diagnosed after the receipt of the provisional criteria.

In the prospective survey, when the interval for reassessment proposed by the provisional criteria was compared with the actual interval recorded, the actual interval was shorter than that proposed by the provisional criteria in 2 cases, and longer in 12 cases. That is, among 6 neonates and infants, the actual interval agreed with the provisional criteria in one neonate (48

hours), and 3 of 5 infants (24 hours), and was longer in 2 cases; among 14 young children, agreement with the provisional criteria (12 hours) was found in only 2 children; the actual interval was longer than that proposed in the provisional criteria in 10 cases. Moreover, the interval was longer than 24 hours in 5 of the 10 cases. It would be reasonable to conclude from these results that the interval of 12 hours for reassessment proposed by the provisional criteria in young children is not a very practical one in the actual clinical setting. In the present study, in all the cases in Groups I–III that were subjected to two or more assessments (regardless of the performance/non performance of the apnea test), the results of the assessments were consistent, regardless of the interval. All of these cases eventually developed cardiac arrest. There were no evident relationships between the patient age or the cause of brain death and the interval to reassessment.

5) Reassessment in Group I

Intervals to reassessment were examined in the 20 cases of Group I. The interval was under 12 hours in 2 cases (10%), under 24 hours in 16 cases (80%), and under 48 hours in 18 cases (90%). Thus, the interval was under 24 hours in most cases, and exactly 24 hours in 10 cases (50%).

6) Pupillary diameter during the observation period

The results of assessment of the state of deep coma and the brainstem reflexes were consistent in the first, second and third (if applicable) assessments in all cases. When the standard pupillary diameter was set at 4.0 mm or more for both the sides,[17,18] the pupillary diameter varied from the first to the second assessment in 23 out of 95 cases. Of these 23 cases, the pupillary diameter was reduced in 10 cases, even though the actual diameter itself was greater than 4 mm. In the remaining 13 cases, the pupils were dilated. The range of variation was 0.5–2.0 mm. Overall, the results of judgment of pupillary dilatation were consistent.

(8) Interval between the diagnosis of brain death and the occurrence of cardiac arrest

In the present study, all the cases eventually developed cardiac arrest without showing any improvement. Table 7 shows the time interval between the diagnosis of brain death and the occurrence of cardiac arrest. The subjects for this analysis were restricted to those cases from each group for whom the records showed that mechanical ventilation was discontinued immediately after the development of cardiac arrest (116 cases in total). About 65–90% of cases in each group developed cardiac arrest within 30 days after the diagnosis of brain death.

In Groups I and II, more than 50% of cases developed cardiac arrest within 8–14 days of the diagnosis of brain death. The results were similar in Group III. In Group IV, however, more than 50% of the cases developed cardiac arrest within a day after the diagnosis of brain death. On the other hand, in 8 (29%) of 28 cases in groups I and II, more than 30 days elapsed before cardiac arrest occurred after the diagnosis of brain death; the corresponding percentages were 25% (14/56 cases) in Group III and 9% (3/32) in Group IV.

(9) Autopsy

Autopsy was performed in 15 cases. Judicial autopsy or administrative autopsy was performed in 5 of these 15 cases. However, no detailed information had been obtained yet as of the time of this report. Among the remaining 10 cases, autolysis of the brain was recorded in 6 cases, brain edema in 3, intracranial bleeding in 3, and cerebral herniation in 1.

In Group I, autopsy was performed in 3 cases, and autolysis of the brain was recorded in 2 of these 3 cases.

(10) "Long-term" brain death

1) Definition of "long-term" brain death

According to the MHW study,[1] the mean

Table 7 Time Until Cardiac Arrest after Diagnosis of Brain Death (%)

Group / Observation period	I+II		I		II		III		IV	
	No. of cases	Cumulative No. of cases	No. of cases	Cumulative No. of cases	No. of cases	Cumulative No. of cases	No. of cases	Cumulative No, of cases	No. of cases	Cumulative No. of cases
0 days	0	0 (—)	0	0 (—)	0	0 (—)	0	0 (—)	7	7 (22)
1 day	3	3 (11)	2	2 (10)	1	1 (13)	2	2 (4)	10	17 (53)
2 days	0	3 (11)	0	2 (10)	0	1 (13)	2	4 (7)	4	21 (66)
3 days	0	3 (11)	0	2 (10)	0	1 (13)	6	10 (18)	4	25 (78)
4 days	4	7 (25)	2	4 (20)	2	3 (38)	3	13 (23)	0	25 (78)
5 days	1	8 (29)	1	5 (25)	0	3 (38)	2	15 (27)	0	25 (78)
6 days	2	10 (36)	1	6 (30)	1	4 (50)	2	17 (30)	3	28 (88)
7 days	0	10 (36)	0	6 (30)	0	4 (50)	3	20 (36)	0	28 (88)
8–14 days	5	15 (54)	4	10 (50)	1	5 (63)	12	32 (57)	0	28 (88)
15–21 days	2	17 (61)	0	10 (50)	2	7 (88)	7	39 (70)	1	29 (91)
22–29 days	3	20 (71)	3	13 (65)	0	7 (88)	3	42 (75)	0	29 (91)
30–99 days	4	24 (86)	3	16 (80)	1	8 (100)	9	51 (91)	2	31 (97)
100 days or more	4	28 (100)	4	20 (100)	0	8 (100)	5	56 (100)	1	32 (100)

Note: A total of 116 cases whose medical records specified that mechanical ventilation had been discontinued after the occurrence of cardiac arrest were included in the analysis.

time interval between brain death and cardiac arrest in adults was 4.3 days. It was reported that 95% of patients died of cardiac arrest about 4 days after the diagnosis of brain death. The analysis included 718 cases, 57 of whom were children under 16 years of age. Among these, in 26 children younger than 6 years, the mean time interval between brain death and the occurrence of cardiac arrest was 11.6 days. There were 20 cases in whom the vital functions were maintained for more than 15 days, and 3 of these were children under 6 years of age. It is evident from the aforementioned that the interval between the diagnosis of brain death and the eventual occurrence of cardiac arrest tends to be longer in children.

In the present study, 116 cases for whom records specified that mechanical ventilation was discontinued immediately after the development of cardiac arrest were chosen from the

total of 139 cases for analysis. A case with prolonged brain death was defined as one in which the interval between the diagnosis of brain death and the occurrence of cardiac arrest was 30 days or longer.

2) Comparison of "long-term" brain death with non-prolonged brain death

In the present study, all cases eventually developed cardiac arrest without any improvement in brain functions. Of all the cases, 25, in whom 30 days or longer elapsed before the eventual development of cardiac arrest were compared with the 91 cases in whom the interval was shorter, in regard to the background factors, cause of disease, and course after brain death until the development of cardiac arrest. Differences were tested by the chi-squire test, and numerical data were tested by the *t*-test, with the significance level set at $p<0.05$.

There were no significant differences between the two groups in regard to the gestational period, body weight at birth, age of the child, age at onset of causal event and, time interval between onset of causal event and the diagnosis of brain death.

i) Institutions: Twenty-one out of 25 cases of prolonged brain death were admitted in pediatric departments. This figure was statistically significantly higher than the other departments.

ii) Type of survey: Prolonged brain death occurred in 8 (40%) of the 20 cases in the prospective survey and in 17 (18%) of the 96 in the retrospective survey, revealing a significantly higher incidence in the cases in the prospective survey. This may be attributable to all of the prospective survey cases being recent cases (from May 1998 onward), and it is possible that advances in patient management had an influence.

iii) Cause of brain damage: The most frequent cause of brain damage in prolonged brain death cases was acute encephalopathy, accounting for 28% of the cases (7 cases). On the other hand, head trauma accounted for 35% (32 cases) of those in whom cardiac arrest developed in less than 30 days after the diagnosis of brain death. Among the cases with prolonged brain death, primary brain damage, such as head trauma and cerebrovascular disease, accounted for 3 cases, and acute encephalopathy, for 7 cases. In contrast, secondary brain damage was more frequent, accounting for 15 cases (6 cases with hypoxia, 5 cases with suffocation, and 4 cases of drowning).

iv) Test items: There were no significant differences between the two groups in terms of pupillary diameters of 4 mm or greater, absence of brainstem reflexes, flat EEG, the number of the apnea test sessions, and the incidence of negative results.

v) Spinal reflex: Positive spinal reflex was recorded in 9 (64%) out of 14 cases with prolonged brain death. In contrast, this reflex was positive in only 9 (20%) out of 45 cases in whom the interval between the diagnosis of brain death and the development of cardiac arrest was shorter than 30 days. Thus, a positive spinal reflex was significantly more frequent in the former group. It remains unclear from the present study as to whether or not this can be explained simply by the longer observation period in the former group.

vi) Ancillary tests: Cranial CT, cranial MRI, cranial ultrasonography and supplementary examinations (brainstem auditory evoked potentials, transcranial Doppler ultrasound flowmetry, RI cerebral blood flowmetry, and angiographic cerebral blood flow examination) were performed at similar frequencies in both the groups, and the results were also similar in the two groups.

Comments

The present study represents the first national survey that was conducted for investigating the

actual status of diagnosis of brain death in children in Japan. It had been believed that reports of brain death in children would be difficult to come by, as a 6-month national survey by the MHW (total 718 cases) in 1984 revealed only 26 reports of brain death in children younger than 6 years of age, the percentage being as low as 3.6%.[1] However, in the present study, we were able to collect 139 cases, which is comparable to the numbers in corresponding reports from overseas.[19–22]

If one were to consider the authenticity of the data, it would be desirable for this type of study to have a prospective design. However, such studies have been rare even internationally. This could be attributable, in part at least, to the fact that clinical policies have already been established in other countries, and there is scarcely any need to validate the criteria of brain death prospectively. Clinical experience with pediatric brain death is difficult to collate in Japan, which has few pediatric ICUs or pediatric emergency divisions.[23,24] In fact, about half of the respondents in the present study were general pediatric departments, and a limited number of institutions reported multiple cases despite the survey period being as long as about 10 years.

The present study included 30 cases (Groups I and II) in whom the apnea test was performed. Among these, 20 cases (Group I) were diagnosed after repeated rigorous testing, almost completely in accordance with the provisional criteria. When these cases, including infants, were analyzed, there was a divergence between the proposed interval for reassessment and the actual observation period recorded. However, it is technically possible to assess brain death in children according to the provisional criteria.

In the remaining cases (Groups III and IV), although deep coma and clinical apnea were confirmed, the apnea test was not performed. These cases may not be considered as brain-dead cases in the strict sense, and should be clearly distinguished from other cases. However, we considered that the information from these cases might also be useful for investigating the clinical status of diagnosis of brain death.

After the report of the President's Commission of the United States was published in 1981, there has been a worldwide consensus through active discussion, as to the criteria for brain death, diagnosed basically on the finding of deep coma, absence of brainstem reflexes, and confirmation of apnea, which were considered to represent an irreversible and loss of whole brain functions. This is also true for pediatric brain death.[26–30] However, concerning the specific criteria and methods of diagnosis, there are certain differences in details among various countries. For instance, the same criteria are used for both adult and pediatric cases in some countries, while specific tests are added or excluded for pediatric cases in other countries. It is with this background that the present survey was conducted.[31,32]

The provisional criteria provided by this study group were, in general, consistent with the MHW criteria, and differed only in subject grouping, exclusion criteria, and interval for reassessment. This is because we believed that it would first be necessary to determine whether the diagnosis according to the existing legal criteria, i.e., the criteria of the MHW, would be feasible, and to consider modifications of the clinical criteria for brain death in the pediatric age group if this survey revealed difficulty in performing certain examinations, or the need to add other examinations in pediatric cases.

There have been few reports comparing the clinical, including that of the apnea test incorporated in the present provisional criteria, with the results of cerebral blood flow examinations, etc. Flowers *et al.*,[33] in a retrospective study using radionuclide examination of cerebral blood flow in 219 cases, reported that no cerebral blood flow could be demonstrated in 71 cases, including adult cases, in whom brain death was diagnosed in compliance with the clinical criteria for brain death, including the apnea test (12 cases were 5 years old or

younger). Thus, according to them, the accuracy of the clinical diagnosis of brain death was 100%. Shimizu *et al.*[34] carried out a retrospective review of medical records of 228 cases of brain death in children in a pediatric hospital in Toronto between 1990 and 1999. They reported that the cerebral blood flow had stopped or decreased to the threshold level in all of the 27 cases in whom radionuclide examination of cerebral blood flow was performed, whether or not all of the clinical criteria for the diagnosis of brain death were satisfied. Thus, they reported the validity of the prescribed clinical criteria for the diagnosis of brain death in the pediatric age group.

The present provisional criteria proposed by our study group are in accordance with the criteria proposed by the MHW,[2] which were laid down for children aged 6 years or older and adults. However, the provisional criteria are rather stringent for the diagnosis of brain death in pediatric cases, from the international point of view.[35] Haupt *et al.*[36] and Thomke *et al.*[37] compared the criteria for the diagnosis of brain death in various European countries, and found that these criteria were basically consistent, including in regard to the significance attached to the apnea test, but vary somewhat (from nil to multiple use) in the performance of instrumental examinations, such as EEG. Lynch *et al.*[38] also studied the actual status of diagnosis of brain death in the US, and reported that application of clinical criteria alone was the most widespread practice. The Canadian guidelines for the diagnosis of pediatric brain death,[39] the newest available at present, also place much weight on the clinical diagnosis, and are basically the same as our provisional criteria; the items of assessment were also similar. However, the Canadian guidelines do not always demand confirmation of electrical inactivity of the brain for the diagnosis of brain death, and the number of different brainstem reflexes to be tested is smaller. In addition, the Canadian guidelines permit the use of other ancillary tests, including cerebral blood flow examination, when clinical diagnosis is difficult.

An interval of 24 hours is recommended for reassessment when secondary brain damage is the cause of brain death, regardless of the patient's age. In other cases, shorter intervals for reassessment are allowed. However, the Canadian guidelines are different from our provisional criteria in that premature infants (less than 37 weeks of gestation) and neonates less than 7 days old are excluded, and that the use of cerebral blood flow examinations as well as clinical examination is recommended for infants less than a year old. These differences stem from the fact that the provisional criteria proposed by us were prepared for the purpose of investigating the actual status of diagnosis of brain death in Japan, and no age-related exclusions were proposed. These features should be taken into consideration when the actual criteria for the diagnosis of brain death in the pediatric group are developed in Japan.

It should be noted that cases with prolonged brain death (in whom at least 30 days elapsed before the occurrence of cardiac arrest), which had been rare among previously reported adult cases, accounted for nearly 20% of all the cases analyzed in the present study. This situation was often associated with encephalitis or hypoxemia. In particular, there were 2 cases in whom the time interval to the development of cardiac arrest after the diagnosis of brain death was 300 days, no inconsistency with the features of brain death were noted, and autolysis and necrosis of brain tissue were noted on diagnostic imaging or autopsy.[40,41] With advances in intensive care, it is currently possible to maintain the heart beat for long periods of time after the diagnosis of brain death, as long as the patient's general condition is well-maintained by respiratory and circulatory and nutritional management, and infection is successfully prevented.[42,43] Although the occurrence of prolonged brain death is known, reports from overseas are rare. Further investigation is awaited for determining whether such a condition, i.e.,

prolonged brain death, is more characteristic of brain death in the pediatric age group, or is a reflection of current medical practice in Japan where life-prolonging treatment is administered even in hopelessly ill patients if the family so wishes, and where definite criteria for pediatric brain death are not yet in place.

The results of analysis of the present cases and a review of the relevant literature indicate that it is highly feasible to diagnose irreversible loss of brain function in children younger than 6 years old according to the proposed provisional criteria developed on the basis of the ideas proposed by the MHW. It is thus practical to consider specific criteria for the diagnosis of brain death in the pediatric age group.

REFERENCES

1) Project of Special Research Supported by Health Science Research Grant of the Ministry of Health and Welfare: *1984 Report of the Brain Death Study Group*. 1985. (in Japanese)
2) Project of Special Research Supported by Health Science Research Grant of the Ministry of Health and Welfare: *1985 Report of the Brain Death Study Group*. 1986. (in Japanese)
3) Coleman, J.M., Mammel, M.C., Reardon, C. *et al.*: Hypercarbic ventilatory responses of infants at risk for SIDS. *Pediatr Pulmonol* 1987; 3: 226–230.
4) Kahn, A., Rebuffat, E., Sottiaux, M. *et al.*: Management of an infant with an apparent life-threatening event. *Pediatrician* 1988; 15(4): 204–211.
5) Ota, T., Waga, S., Handa, H. *et al.*: New grading of level of disordered consciousness. *Noshinkeigeka* 1974; 2: 623–627. (in Japanese)
6) Teasdale, G. and Jannett, B.: Assessment of coma and impaired consciousness. A practical scale. *Lancet* 1974; 2(7872): 81–84.
7) Morray, J.P., Tyler, D.C., Jones, T.K. *et al.*: Coma scale for use in brain-injured children. *Crit Care Med* 1984; 12(12): 1018–1020.
8) Outwater, K.M. and Rockoff, M.A.: Apnea testing to confirm brain death in children. *Crit Care Med* 1984; 12(4): 357–358.
9) Rowland, T.W., Donnelly, J.H. and Jackson, A.H.: Apnea documentation for determination of brain death in children. *Pediatrics* 1984; 74(4): 505–508.
10) Maekawa, T., Sadamitsu, T. and Tateishi, A.: From the scene of diagnosing brain death: Apnea test. *Rinsho Shinkei* 1993; 33(12): 1331–1333. (in Japanese)
11) Villani, A., Onofri, A. and Bianchi, R.: Determination of brain death in intensive pediatric therapy. *Pediatr Med Chir* 1988; 20(1): 19–23.
12) Bohatyrewicz, R., Kojder, I., Krawczyk, L. *et al.*: Spinal reflexes in the determination of brain death. *Neurol Neurochir Pol* 1997; 31(2): 281–285. (in Polish)
13) Ulvik, A., Salvesen, R. and Niellsen, E.W.: Reflexes in brain-dead patients. *Tidsskr Nor Laegeforen* 1988; 118(13): 2025–2026. (in Norwegian)
14) Wiener, M.D., Garay, S.M., Leitman, B.S. *et al.*: Imaging of the intensive care unit patient. *Clin Chest Med* 1991; 12(1): 169–198.
15) Tobin, J.R., Spurrier, E.A. and Wetzel, R.C.: Anaesthesia for critically ill children during magnetic resonance imaging. *Br J Anaesth* 1992; 69(5): 482–486.
16) Rotello, L.C., Radin, E.J., Jastremski, M.S. *et al.*: MRI protocol for critically ill patients. *Am J Crit Care* 1994; 3(3): 187–190.
17) Ropper, A.H., Kennedy, S.K. and Russell, L.: Apnea testing in the diagnosis of brain death. Clinical and physiological observations. *J Neurosurg* 1981; 55(6): 942–946.
18) Isenberg, S.J., Molarte, A. and Vazquez, M.: The fixed and dilated pupils of premature neonates. *Am J Ophthalmol* 1990; 110(2): 168–171.
19) Mejia, R.E. and Pollack, M.M.: Variability in brain death determination practices in children. *JAMA* 1995; 274(7): 550–553.
20) Parker, B.L., Frewen, T.C., Levin, S.D. *et al.*: Declaring pediatric brain death: Current practice in a Canadian pediatric critical care unit. *CMAJ* 1995; 153(7): 909–916.
21) Keenan, S., Busche, K., Chen, L. *et al.*: A retrospective review of a large cohort of patients undergoing the process of withholding or withdrawal of life support. *Crit Care Med* 1997; 25(8): 1324–1331.
22) Lopez-Herce, J., Sancho, L. and Martinon, J.M.: Study of paediatric intensive care units in Spain. Spanish Society of Paediatric Intensive Care. *Intensive Care Med* 2000; 26(1): 62–68.

23) Hatori, F., Miyasaka, K., Uchida, H. *et al.*: Paediatric brain death viewed from the standpoint of paediatric intensive care in Japan. *Noshi/Nososei Kenkyukaishi* 1999; 12: 39–41. (in Japanese)
24) Barker, G.: Diagnosis of brain death in pediatric critical care patients. *Noshi/Nososei Kenkyukaishi* 1999; 12: 27–38.
25) Van Norman, G.A.: A matter of life and death: What every anesthesiologist should know about the medical, legal, and ethical aspects of declaring brain death. *Anesthesiology* 1999; 91(1): 275–287.
26) American Academy of Pediatrics Task Force on Brain Death in Children: Guidelines for the determination of brain death in children. *Neurology* 1987; 37(6): 1077–1078.
27) Pallis, C. and Harley, D.: *ABC of Brainstem Death*. 2nd ed, BMJ Publishing Group, London, 1996.
28) Ashwal, S. and Schneider, S.: Brain death in the newborn. *Pediatrics* 1989; 84(3): 429–437.
29) Ashwal, S. and Schneider, S.: Pediatric brain death: Current perspectives. *Adv Pediatr* 1991; 38: 181–202.
30) Ashwal, S.: Brain death in the newborn. Current perspectives. *Clin Perinatol* 1997; 24(4): 859–882.
31) ten Velden, G.H. and van Huffelen, A.C.: Brain death criteria; Guidelines by the Public Health Council. *Ned Tijdschr Geneeskd* 1997; 141(2): 77–79. (in Dutch)
32) Ad Hoc Committee of Brain Death of Children's Hospital of Boston: Determination of brain death. *J Pediatr* 1987; 110(1): 15–19.
33) Flowers, W.M. Jr. and Patel, B.R.: Accuracy of clinical evaluation in the determination of brain death. *South Med J* 2000; 93(2): 203–206.
34) Shimizu, N., Shemie, S., Miyasaka, E. *et al.*: Validation of diagnostic criteria for paediatric brain death by radionuclide blood flow examination. *Masui* 2000; 49(10): 1126–1132. (in Japanese)
35) Betschart, M.: The diagnosis of brain death. Guidelines in Germany, Austria and Switzerland. *Aneasthesist* 1993; 42(4): 259–269. (in German)
36) Haupt, W.F. and Rudolf, J.: European brain death codes: A comparison of national guidelines. *J Neurol* 1999; 246(6): 432–437.
37) Thomke, F. and Weilemann, L.S.: Current concepts in diagnosing brain death in Germany. *Med Klin* 2000; 95: 85–89.
38) Lynch, J. and Eldadah, M.: Brain-death criteria currently used by pediatric intensivists. *Clin Pediatr* (Phila) 1992; 31(8): 457–460.
39) Canadian Neurocritical Care Group: Guidelines for the diagnosis of brain death. *Can J Neurol Sci* 1999; 26(1): 64–66.
40) Kuboyama, K., Yoshinaga, K., Marukawa, S. *et al.*: An infantile case of brain death lasting for more than 300 days. *Nippon Kyukyuigakkai Zasshi* 2000; 11: 338–344. (in Japanese)
41) Maeda, M., Miura, S., Okamoto, S. *et al.*: A male infantile case of brain death agreeing with the provisional criteria for diagnosis of paediatric brain death and having continued for about 11 months. *Noshi/Nososei Kenkyukaishi* 1999; 12: 44–45. (in Japanese)
42) Shewmon, D.A.: Chronic "brain death": meta-analysis and conceptual consequences. *Neurology* 1998; 51(6): 1538–1545.
43) Fisher, J.: Re-examining death: Against a higher brain criterion. *J Med Ethics* 1999; 25(6): 473–476.

Progress in Diagnosis and Treatment of Osteoporosis

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Key words: Osteoporosis; Diagnosis; Treatment; BMD; Fracture

New Diagnostic Criteria for Primary Osteoporosis (Revised in 2000)

In 1995 the Japanese Society for Bone and Mineral Research established a committee for study of the diagnostic criteria for osteoporosis consisting of representatives from orthopedic surgery, internal medicine (geriatrics), gynecology, radiology and sports medicine who are engaged in medical care and research of osteoporosis and proposed the diagnostic criteria for primary osteoporosis.[1)] The criteria were reviewed in 1996 and the 1996 revision was published in 1996.[2)] The 2000 revision was published by incorporating the new findings in osteoporosis studies conducted since 1996.[3)]

1. Definition of osteoporosis

Osteoporosis is defined as pathological reduction in bone mass and changes in microstructure of bone tissues, making bones both fragile and easily fractured.

2. Basic philosophy underlying diagnostic criteria for primary osteoporosis

Primary osteoporosis is diagnosed based on the findings of vertebral X-ray and bone density measurement.

1) Reduction in bone mass may be evaluated either by vertebral X-ray or measurement of bone density, but the latter is employed as a rule. Vertebral X-ray is relied upon when it is difficult to measure or evaluate the bone density.
2) Differential diagnosis is performed for subjects with fragile bone fracture or those whose bone density is 70% or less than that of the average young adults (YAM) to exclude diseases other than osteoporosis that cause reduction of bone mass.
3) After differential diagnosis, osteoporosis is diagnosed using the diagnostic criteria. In other words, diagnosis is made by exclusion.

Several problems have been pointed out since the proposal of the diagnostic criteria in 1996. In view of an opinion that the criteria should be re-examined in regard to their validity, the committee took up the issue.

According to the 1996 criteria, cut-off value of the bone density that can efficiently distinguish those with and without spinal fracture based on specificity and sensitivity was determined using the data of cross sectional study of bone mineral density. At this time, a longitudinal study was conducted to examine whether or not the cut-off value was valid. The result

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Table 1 Diagnostic Criteria for Primary Osteoporosis (2000 Revision)

Primary osteoporosis is diagnosed when diseases other than osteoporosis that induces reduction in bone mass or secondary osteoporosis are not recognized and the result of bone assessment satisfies the following conditions.

I. Presence of fragile bone fracture[NB1)]		
II. Absence of fragile bone fracture		
	Bone density[NB2)]	Osteoporotic changes in spinal X-ray[NB3)]
Normal	≧80% of YAM	Absent
Reduction in bone mass	80%＞YAM≧70%	Suspected
Osteoporosis	＞70%	Present

YAM: Young adult mean (20–44)

NB 1) Fragile bone fracture: non-traumatic bone fracture caused by slight external force due to reduced bone mass (BMD is less than 80% of YAM, or osteoporotic changes are present in spinal X-ray). Fractured sites are the spine femoral neck, distal end of the radial, etc.

NB 2) BMD is, as a rule, measured at the lumbar spine. If it is judged inappropriate, however, to measure BMD of the lumbar spine because of spinal deformity, etc. in the elderly, BMD of the femoral neck is measured. If measurement of BMD at these two sites are difficult, BMD of the radius, second metacarpal bone or ankle bone is used.

NB 3) Evaluation of osteoporotic changes by spinal X-ray is made by referring to the conventional criteria for determination of bone atrophy.

Osteoporotic changes as observed by spinal X-ray	Conventional criteria for determination of bone atrophy
Absent	Atrophy absent
Suspected	Bone atrophy, degree I
Present	Bone atrophy, ＜degree II

revealed that the cut-off value was 0.737 g/cm^2 (72.9% of YAM), which was slightly different from the cut off value of 0.760 g/cm^2 (75.2% of YAM) obtained from the cross sectional study of the same subjects. The difference, however, was within the allowable range of errors and was concluded as substantially the same. The above result based on the longitudinal study clearly demonstrated that the cut-off value based on the result of 1996 cross sectional study was valid.

3. New diagnostic criteria for primary osteoporosis (Women) (2000 Revision) (Table 1)

The committee examined various problems of the diagnostic criteria (1996 revision) and established a new diagnostic criteria (2000 revision) shown in Table 1.

4. Diagnostic criteria for primary osteoporosis in men

In order to study the above-mentioned issue, a multi-institutional cross sectional study was performed on Japanese men aged 60 and older.

The cut-off values for BMD (bone mineral density) of the femoral neck and the lumbar spine were set to discriminate the presence or absence of fracture of the femoral neck or the lumbar spine. In men, BMD of the femoral neck was more useful than that of lumbar vertebrae for discriminating the presence or absence of fracture. The cut-off value of BMD of the femoral neck was 73.6% of YAM and that for the lumbar spine 72.8%, indicating close proximity of the two values. Based on the above, it is considered that no change is needed for the diagnostic criteria for men from those for women.

Table 2 Large-scale Clinical Trial with Vertebral Pyramid as End Point

Large-scale clinical test (Reference)	Increase ratio of vertebral BMD	Decrease ratio of vertebral fracture	Vertebral T score	Pyramid fracture
FIT II	8.3%	44%	−2.1	0%
FIT I	7.9%	47%	−2.5	100%
RVE	7.1%	49%	−2.8	100%
RVN	5.4%	41%	−2.4	100%
MORE	2.6%	40%	−2.6	37%
PROOF	1.2%	36%	<-2.0	100%

FIT I and FIT II are administered alendronate, RVE and RVN risedronate, MORE raloxifene and PROOF carcitonin
(From Faulkner, K.G.: *J Bone Miner Res* 2000; 15: 183)

Current Status and Challenges in the Treatment of Osteoporosis

In 1998, the working group for the study of osteoporosis of longevity science foundation evaluated the information on the therapeutic agents for osteoporosis that are currently covered by the health insurances for reimbursement, and proposed the guidelines for pharmaceutical therapy of osteoporosis. Numerous large-scale clinical studies have been conducted in western countries during the subsequent four years and many new evidences have been presented. It is extremely regrettable, however, that introduction of the new GCP (good clinical practice) to Japan have changed the system of drug developments radically, and consequently few large-scale studies have been conducted in this country.

Therefore I would like to review the major study results obtained in western countries based on the principle of evidence based medicine (EBM), and make a comment as regards the future prospects.

1. Does drug administration to increase bone mass effectively prevent fractures? — demonstration by multi-institutional double blind study —

Many studies demonstrate that the bone mass is a principal factor in defining bone strength. It is generally held by many prospective studies that 60–80% of bone strength is explainable by BMD and that low BMD is a risk factor for bone fracture. With lowering of BMD, risk for bone fracture increases as exponential function. Does it mean then that increasing BMD by drugs, etc. prevents fractures? Based on such a viewpoint, many clinical studies have been performed in the west with prevention of fracture as an end point of evaluation.

Table 2 shows the results of large-scale clinical studies with bone fracture as an end point.[4] FIT I and FIT II show the ratios of increase in spinal BMD and decrease of spinal fractures when alendronate is administered, RVE and RVN when risedronate is administered, MORE when raloxifene is administered, and PROOF when calsitonin is administered (compared to placebo groups) respectively for three years. Although there are differences in BMD increase and fracture prevention depending on drugs, it is apparent that the increased bone mass by drug contributes to prevention of spinal fractures.

2. Effects of physical exercises as examined by meta-analysis

Focus is currently placed on the relation between physical exercises and BMD, and the results of many studies have been published. These study results, however, are mixed in their

merit or quality and many are not worthy of review from the viewpoint of EBM (evidence based medicine) since only a limited number of cases were studied for only a short period of time. Under such circumstances, it may be a useful strategy to gather results of several finer reports and conduct meta-analysis. There is a study reporting the result of meta-analysis of 5 to 16 randomized controlled trials that examined the effects of physical training on BMD of the lumbar vertebrae or the femoral neck.[5] According to this study, the increase of BMD by exercises is 0.84%/year for the lumbar vertebrae and 0.89%/year for the femoral neck, indicating that efficacy is relatively inferior compared to drugs.

REFERENCES

1) Orimo, H. *et al.*: *J Bone Miner Res* 1995; 13: 106 (in Japanese)
2) Orimo, H. *et al.*: *J Bone Miner Res* 1996; 14: 219 (in Japanese)
3) Orimo, H. *et al.*: *J Bone Miner Res* 2000; 15: 193 (in Japanese)
4) Faulkner, K.G.: *J Bone Miner Res* 2000; 15: 183.
5) Wolft, I. *et al.*: *Osteoporos Int* 1999; 9: 1.

Health Impact of Dioxins

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Key words: Dioxin; Toxity; Carcinogenicity; TCDD; Risk

Dioxins, as well as endocrine disrupters, are a group of chemicals that have received widespread publicity on a daily basis as new environmental pollutants. They are routinely described as "deadly poisons and the most potent carcinogens" resulting in fear and anxiety in the public at the mention of dioxins.

Various animal studies have shown that the lethal dose median (LD_{50}) of dioxins in guinea pigs is 1μg/kg body weight, indicating that the estimated toxicity of dioxins is 60,000 times higher than that of potassium cyanide. Long-term animal studies with dioxins have also demonstrated its carcinogenicity, teratogenicity, immunotoxicity, and reproductive toxicity. These data certainly indicate that dioxins are deadly poisons.

The concentration of dioxins is extremely low both *in vivo* and in the environment. The blood concentration of dioxins in the public is in the order of picograms per gram fat (pg; one trillionth of 1 g) and is much lower than that of other general environmental pollutants that are present in the order of milligrams per gram fat (mg; one thousandth of 1 g). In animal studies, dioxins are routinely used at very low doses of 1μg/kg body weight per day, whereas other common types of chemical toxins are usually used at a dose of 1 mg/kg/day. Since various harmful effects have been observed even with this low dose, dioxins have the reputation of being deadly poisonous. However, although the dose of 1μg/kg body weight per day, i.e. one thousandth of a gram appears small, it is not so small when viewed from another standpoint. It is one million times greater than 1 pg and 250,000 times greater than 4 pg/kg, the daily intake of dioxins in the public.

General Toxicity of Dioxin in Humans

An epidemiological study has been conducted recently in those involved in the accidental explosion at an agricultural chemical plant in Seveso, Italy, workers in the agricultural chemical manufacturing industry, and Vietnam veterans, in order to investigate the acute and chronic effects of dioxin exposure. The cumulative number of subjects was ca. 340,000, and the follow-up period was from 15 to 50 years. Surprisingly, no cases of acute poisoning have been reported in the study to date.

In 10 children living in Zone A, the Zone where subjects were exposed to the highest level of dioxins in Seveso, the blood level of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; the most toxic compound in the dioxin family) immediately after exposure ranged from 828 to 56,000 pg/g fat. This is equivalent to 202 to 13,659 times the normal level found in the public.

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Table 1 Toxicity Parameters for Dioxin (TCDD) in Humans Calculated by Extrapolation of Toxicity Data in Animals

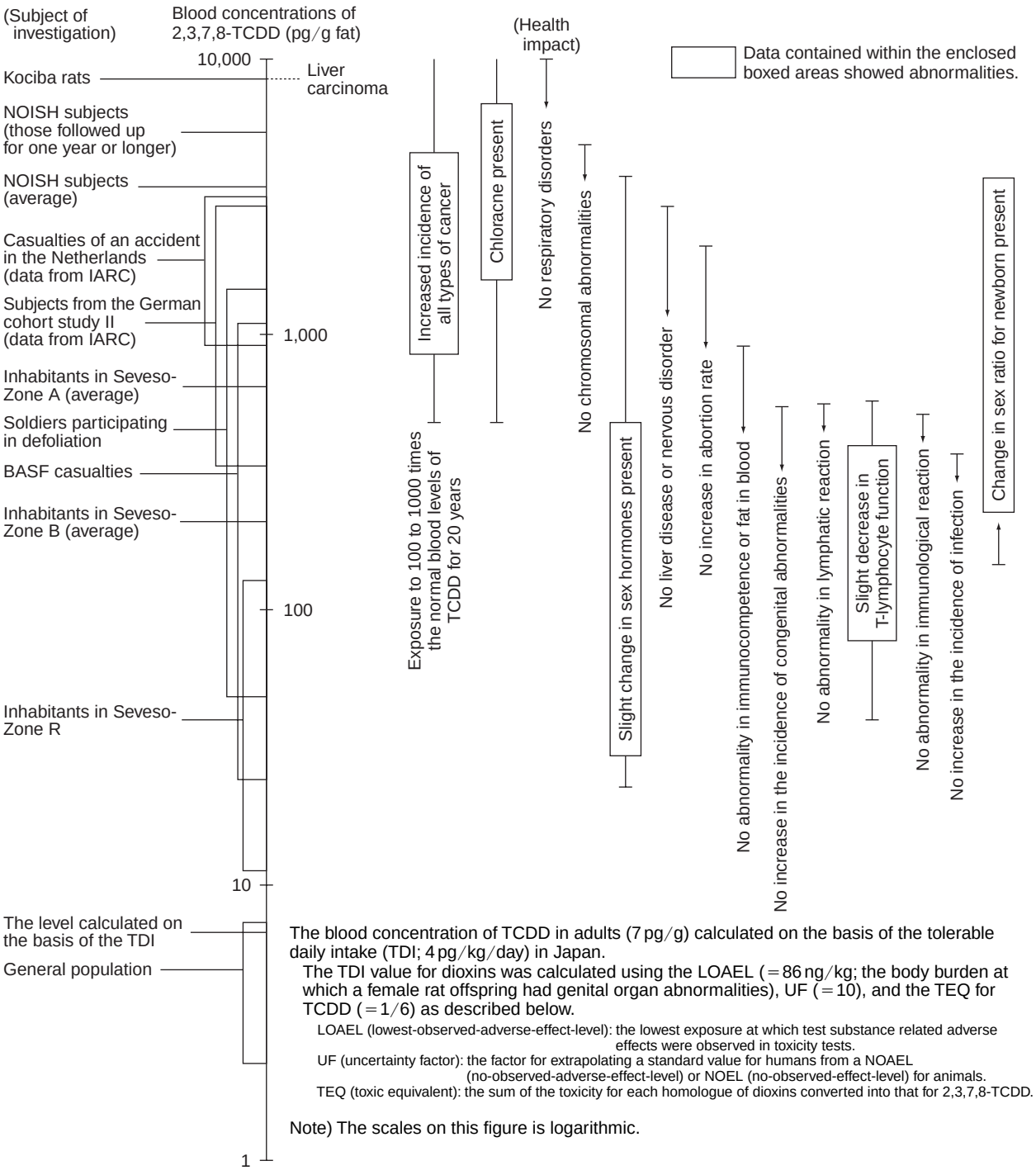
	Species	Acute toxicity	Chronic toxicity or RfD		Usefulness (?)
			Long-term mortality	Carcinogenicity	
Animals	Guinea pigs	$LD_{50} = 1\mu g/kg$ (deadly poison)	Estimated RfD in humans = 1 pg/kg/day = the blood level of 10 pg/g fat		
Animals	Rats	LD_{50} (in male) $= 22\mu g/kg$	Estimated RfD in humans = 22 pg/kg/day = the blood level of 220 pg/g fat	Liver carcinoma following exposure to 100 ng/kg/day NOAEL = 1 ng/kg/day = TDI in humans = 10 pg/kg/day = the blood level of 100 pg/g fat	Protective activities at 43–10,000 pg/kg/day (anti-cancer, etc. in rats, mice, and marmosets)
Humans	People exposed to dioxins in Seveso	Short-term intake (children in Zone A) 2.5μg/kg on average, 8.4μg/kg in maximum No acute toxicity	15-year follow-up in the 40,000 inhabitants of Seveso No increase in mortality	Blood level of 5–400 pg/g fat No increase in incidence of cancer	In the inhabitants in Zone R (with the blood level of 11–118 pg/g) and men in Zone A (having higher exposure), the incidence of cancer decreased.
Humans	Agricultural chemical manufacturers, etc.	Blood level of 210–56,000 pg/g fat No acute poisoning Chloracne present	Of 20 studies with follow-up periods of 15–50 years in 340,000 people with a blood TCDD level of 38–56,000 pg/g, only one study showed a significantly elevated risk of death of 1.3 times the normal rate after 20-year exposure. No increase was noted in the other 19 studies.	Only in a population with a 100–1,000 times higher blood level than that in the public (400–4,000 pg/g), the risk significantly increases by 1.4 times after 20-year exposure.	

Since the human is less sensitive to dioxins than animals, the safe dose in humans calculated from that in animal studies via risk assessment becomes overestimated. Prevention should be considered on the basis of risk assessment, while actual countermeasures should be taken on the basis of hazard assessment.

The short-term intake estimated from the above concentration was a maximum of 168,000,000 pg or 8,400,000 pg/kg body weight, corresponding to 2.1 million times higher than the level in the public. However, even at these very high levels of exposure, the only symptom of poisoning observed was chloracne, and laboratory tests revealed no particular abnormalities.

In terms of the long-term mortality, 20 studies have been conducted in 340,000 people with follow-up periods of 15 to 50 years with blood TCDD concentrations of 38 to 56,000 pg/g fat (versus 2 to 8 pg/g fat in the public). Nineteen of these studies demonstrated no significant increase in mortality, and only one study reported a 1.3 fold increase in mortality after 20 years of exposure (Table 1).

In preventive strategies incorporating risk assessment, the level shown to be safe in animals is multiplied by various safety coefficients to calculate the standard reference value in humans (tolerable daily intake [TDI] or risk reference dose [RfD]). To obtain the standard value for TCDD in humans on the basis of the LD_{50} in guinea pigs (1μg/kg), we need to estimate a chronic value from an acute value, a no-



The blood concentration of TCDD in adults (7 pg/g) calculated on the basis of the tolerable daily intake (TDI; 4 pg/kg/day) in Japan.

The TDI value for dioxins was calculated using the LOAEL (= 86 ng/kg; the body burden at which a female rat offspring had genital organ abnormalities), UF (= 10), and the TEQ for TCDD (= 1/6) as described below.

LOAEL (lowest-observed-adverse-effect-level): the lowest exposure at which test substance related adverse effects were observed in toxicity tests.

UF (uncertainty factor): the factor for extrapolating a standard value for humans from a NOAEL (no-observed-adverse-effect-level) or NOEL (no-observed-effect-level) for animals.

TEQ (toxic equivalent): the sum of the toxicity for each homologue of dioxins converted into that for 2,3,7,8-TCDD.

Note) The scales on this figure is logarithmic.

Fig. 1 Health impact as related to blood concentrations of dioxin (2,3,7,8-TCDD) (prepared on the basis of data from numerous reports)

Kociba rats: In this study by Kociba, the rats were fed 2,3,7,8-TCDD for two years and the development of hepatocellular carcinoma was investigated and reported.

NIOSH: National Institute for Occupational Safety and Health, which conducted a large-scale study on occupational exposure to dioxins in U.S. workers in chemical plants.

BASF casualties: Those involved in the accidental explosion at the BASF agricultural chemical plant, in the former West Germany in 1953.

Seveso-Zone A: The area polluted by the highest level of dioxins in Seveso.

Seveso-Zone B: An area less polluted than Zone A.

Seveso-Zone R: An area with a minimum of pollution considered to be less dangerous.

observed-adverse-effect-level (NOAEL) from an intoxicating level, and a value in humans from that in animals, resulting in the respective safety coefficients of 1/100, or the total safety coefficient of 1/1,000,000 at least. Accordingly, the standard value in humans can be calculated as 1 pg/kg/day, corresponding to a blood level of 10 pg/g fat. This result shows that the standard accepted TDI for TCDD in humans as determined by risk assessment is far from the actual situation.

The data above show that dioxins are not actually deadly poisonous to humans.

Carcinogenicity of Dioxins in Humans

A long-term carcinogenicity study in rats demonstrated that the incidence of hepatoma significantly increased after exposure to dioxins at 100 ng/kg/day for two years, and the no-effect toxic dose was 1 ng/kg/day. Multiplying this value by the safety coefficient of 1/100 gives 10 pg/kg/day, the former international TDI. However, in terms of carcinogenesis in humans, a long-term epidemiological study in people living in Seveso has shown no increase in the incidence of total cancers or hepatoma.

The International Agency for Research on Cancer (IARC) has recently accepted that only TCDD of the dioxin family is "human carcinogen". This conclusion was based on results from a number of epidemiological surveys in humans. IARC has concluded that the incidence of cancer is significantly increased only when people are exposed to more than 100 to 1,000 times the normal blood levels of TCDD for 20 years or longer, and that the relative risk is 1.4 times. However, many animal studies and epidemiological surveys in humans have demonstrated that exposure to several times to several hundred times higher TCDD levels than that in the public exhibits an anti-promotion or anti-cancer effect. Therefore, on a scientific basis, dioxins are not considered to be the most potent carcinogen in humans.

Figure 1 illustrates various effects of dioxins on humans on the basis of their concentration in blood. The effect observed at the minimum level is reproductive toxicity, on which additional studies are needed in the future. However, no detrimental impacts on humans have been reported to be linked to exposure to normal or slightly elevated levels of dioxins.

Fortunately, the level of dioxins in the human body has decreased over the last few decades both in Japan and in other countries. At least in Japan, the major reason for higher dioxin levels in the past has been widespread use of dioxin-contaminated pesticides, and dioxins emitted from incinerators have probably not had a great influence on dioxin levels in humans.

It is important that physicians provide people with correct information in order to relieve their anxiety in relation to dioxins.