ポスター発表P－14

Menkes disease, an inborn error of copper metabolism: pathogenetic mechanism and therapeutic strategy

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Key Words : Menkes disease in humans and mice, Inborn error of copper metabolism, Brindled mouse, Copper-dependent enzyme,Copper-injection therapy, Gene therapy

Ⅰ. Background and Purpose

Menkes disease is an X-linked recessively inherited disorder of copper (Cu)
metabolism, characterized by kinky hair, hypocupremia and progressive brain
degeneration. The basic abnormality due to the genetic defect has become evident to be impaired intracellular Cu transport. This results in Cu deficiency of the peripheral blood, brain and liver, and Cu accumulation in other organs such as the intestine and kidneys. In connection with this deficiency, activities of Cu-containing enzymes such as cytochrome oxidase (CCO) and superoxide dismutase (SOD) in tissues are decreased. This has been known to be responsible for most of the clinical manifestations of this disease. Patients die at age 12 months on average, usually after repetitive attacks of epileptic convulsions. The brain is severely atrophied. There is marked atrophy of the cerebral cortex with focal or diffuse neuronal loss, in addition to diffuse atrophy of the white matter. There is also severe atrophy of the thalamus and red nucleus. The cerebellar cortex shows a specific change to this disease, which indicates inhibited development of Purkinje cells, i.e., somatic sprouts and dendritic arborization. A universal change seen in those degenerated lesions is mitochondrial abnormalities which include ballooning, vesiculation of cristae, and sometimes dense body formation.

The brindled mouse is an excellent animal model for Menkes disease in humans. They are equivalent to each other in clinical symptoms, biochemical and pathological changes and causative genes. Animal models are very useful, because they enable us to study what we cannot put into practice with patients. The purpose of the present study is to elucidate the pathogenetic mechanism and develop the therapeutics of Menkes disease by using its mouse model.

Ⅱ. Subjects and Methods

Subjects

1. Non-treated group: 1-day-postnatal to 13-day-postnatal brindled mice (BM) and age-matched normal control male mice (NM)
2. Treated group: individual age groups that could survive by a single subcutaneous injection of CuCl2 (10ug/g(b.w.)) on postnatal day 7.

Methods 1. Light microscopy including enzyme histochemistry and immunohistochemistry.

2. Electron microscopy (JEM2000EX) including immuno-electron microscopy and enzyme-electron microscopy.
3. Electron probe X-ray microanalysis (Hitachi H600 total system electron microscope linked to an X-ray microanalyzer).
4. Enzyme activity assay (CCO and SOD).
5. Determination of element concentrations of various tissues by flameless atomic absorption spectrophotometry (Hitachi AAS 170-70) after low temperature-reducing to ashes.

Ⅲ. Results and Discussion

Progressive ballooning of mitochondria of neurons became evident after postnatal day 7, and many neurons in the cerebral and cerebellar cortices showed marked vacuolar change and BM invariably died on postnatal day 14 or 15.

However, when BM received a single subcutaneous injection of CuCl2 (10ug/g(b.w.)) on postnatal day 7, the effect was so dramatic that the animals became vivid and could survive as long as the NM: the fur became light gray and they could make females pregnant, and there was no neurological or other signs but their whiskers remained kinky. The activity level of CCO of the brain of non-treated BM showed only 30% of that of NM. The enzyme activity of treated BM, however, recovered gradually and reached the normal level full 6 months later.

The same was true for SOD, though its activity level of the brain tissue of BM on postnatal day 13 was approximately 70-80% of that of NM. The enzyme electron microscopy of the brains from non-treated 13-day-old BM revealed marked ballooning of mitocndria with a strikingly reduction of CCO activity. In contrast, that of the brains from 7-month-old BM and age-matched NM demonstrated that there was no difference between the two in the activity level of CCO as well as the morphology of mitochondria. Nevertheless, the recovery of tissue Cu levels of those BM was insufficient: cerebrum 58%, liver 82%, kidney 380%, small intestine 173%, and peripheral blood 89 % in BM/NM ratio, respectively.

Recently, therapy with Cu-histidine injection has modified the severity of Menkes disease and permitted survival into adolescence. However, patients generally cannot live beyond 30 years of age and neurological manifestations cannot be prevented. The difficulties to start Cu-injection therapy as early as it is possible and issues of Cu-intoxication leading to renal failure remain to be solved. Because there is no other way to take over, clinicians even now have to use the Cu-repetitive injection therapy.

The mouse atp7a gene that is analogous to human ATP7A gene has been identified
at the X-linked mottled locus so far. The mutation of this gene causes Menkes disease in mice. Therefore, by infecting retroviral vectors to targeting tissues and cells which mouse Menkes gene was introduced into, it could be possible to cure Menkes disease in mice, because the viral vectors are integrated in chromosomes and the gene is expressed permanently. This gene therapy for mouse Menkes disease will be the herald of those for human Menkes and Wilson diseases.

Acknowledgements

Individual studies were reported previously as joint researches with collaborators (1982-1998). This study, a work of comprehensive survey of our previous studies on Menkes disease in humans and mice, has been made with rearrangement of them. The authors express their heartfelt gratitude to those who supported and helped them as well as to previous collaborators.

References

1. NO TO HATTATSU 15:40-46, 1982
2. J Clin & Exp Med 124:709-711, 1983
3. Acta Neuropathol(Berl) 59:295-303, 1983
4. Neurol Med 25:595-598, 1986
5. Acta Pathol Jpn 38(6):705-712, 1988
6. Acta Pathol. Jpn 40(6):383-390, 1990
7. Neurol Med 35:234-235, 1991
8. J Intellct Disab Res 37:561-567, 1993
9. Pathol Internat 44:14-19, 1994
10. Neuropathology 14:81-90, 1994
11. Pathol Internat 45:10-18, 1995
12. Current Encyclopedia of Pathol (Suppl 3), Nakayama-shoten, Tokyo,1996, pp.223-237
13. Neuropathology 17:313-318, 1997
14. Neuropathology 18:38-46, 1998

演題の日本語訳：

Menkes 病（先天性銅代謝異常症）：発症機序と治療戦略