Short Communication

Mottled gene expression and copper distribution in the macular mouse, an animal model for Menkes disease

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Menkes disease (McKusick 309400) is an X-linked neurodegenerative disorder characterized by copper accumulation in various organs and cells, including the intestine and kidney (Kodama 1996). In this disease, digested copper accumulates in the intestine, resulting in a failure of copper absorption and subsequently a copper deficiency in the blood, liver and brain. The Menkes gene encodes a copper-transporting ATPase (ATP7A) expressed in almost all tissues, including the intestine and kidney, except for the liver in normal humans (Chelly et al 1993; Mercer et al 1993; Vulpe 1993). Mutations in the ATP7A gene of patients with Menkes disease have been reported, suggesting that a defect in ATP7A function results in copper accumulation in the cells. However, little is known about the relation between ATP7A and copper distribution in individual tissues.

The macular mouse is similar to patients with Menkes disease in terms of clinical phenotype and biochemical abnormalities. Recently we identified a missense mutation in the mottled gene (atp7a) of the macular mouse (Murata et al 1997), validating the use of macular mice as a model of Menkes disease. In this study, we examined expression of atp7a in the intestine, kidney and brain of normal and macular mice by in situ hybridization. We also investigated the copper distribution in these tissues and will discuss here the relation between atp7a expression and copper distribution.

MATERIALS AND METHOD

Materials: Male hemizygotic macular mice were treated with a subcutaneous injection of cupric chloride solution (50 μg of CuCl₂) on postnatal day 7. They were kept under standard conditions and then prepared for study. Age-matched male C3H strain mice were used as controls.
**In situ hybridization:** Ether-anaesthetized 4-week-old mice were transcardially perfused with 4% paraformaldehyde, phosphate-buffered saline, pH 7.4. The intestine, kidney and brain were excised, and embedded in OCT compound. Cryostat sections were cut and mounted on glass slides. **In situ hybridization** was carried out as described previously (Murata et al 1997). A 2.3 kb fragment of mottled cDNA corresponding to bp 1819–4163 (Levinson et al 1994) into SKII Bluescript vector was used as a probe. Sense and antisense strands of RNA probes were labelled with digoxigenin-UTP using T7 and T3 RNA polymerase. The probes were reduced to an average size of 150 nucleotides by alkaline hydrolysis. After **in situ** hybridization and washing, these sections were incubated with an alkaline phosphatase-labelled anti-digoxigenin antibody solution. The samples were developed using 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate.

**Preparation of sections for copper staining:** The sections with copper staining for light microscopy were prepared according to a modified sulphide-silver method as described previously (Kodama et al 1993). The procedures are briefly as follows. Ether-anaesthetized mice were transcardially perfused with a solution of 0.3% sodium sulphide and 1.2% sodium phosphate. The intestine, kidney and brain were excised, embedded in OCT compound and then frozen. Cryostat sections (10 μm) were cut and mounted on glass slides. The sections were placed in 15% trichloroacetic acid solution for 15 min, and then stained with a Timm’s solution. The incubation times were 45 and 70 min for the sections of the brain and intestine, and the kidney, respectively.

**RESULTS AND DISCUSSION**

The results obtained concerning expression of the mottled gene and copper staining in the intestine, kidney and brain of normal and macular mice are summarized in Table 1. By **in situ** hybridization, intense signals were observed in the absorptive epithelial cells and Paneth cells of normal intestine, and in the proximal tubular cells of normal kidney. The corresponding cells in macular mice were also labelled, but the intensity of labelling in macular mice was slightly weaker than that of controls. Copper staining studies showed that copper accumulates excessively in the absorptive epithelial cells, Paneth cells and proximal tubular cells of normal mice (Table 1). These results show that ATP7A/atp7a plays a role in copper transport in the intestinal epithelial cells and renal proximal tubular cells, and that a dysfunction of ATP7A/atp7a causes copper to accumulate in these cells. On the other hand, the glomeruli and distal tubular cells in both the control and macular mice showed very low levels of expression of the mottled gene, consistent with the finding that no copper accumulates in these cells of the macular mice (Table 1).

In the brain of normal and macular mice, expression of the mottled gene was most intensely evident in the choroid plexus, in Ammon’s horn and the dentate gyrus in the hippocampus, and in Purkinje cells and the granular layer of the cerebellum. The ependyma, piriform region, amygdaloid body and medial habenular