Increased levels of plasma lysosomal enzymes in patients with Lowe syndrome

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Lowe syndrome is an X-linked disorder that has a complex phenotype that includes progressive renal failure and blindness. The disease is caused by mutations in an inositol polyphosphate 5-phosphatase designated OCRL. It has been shown that the OCRL protein is found on the surface of lysosomes and that a renal tubular cell line deficient in OCRL accumulated substrate phosphatidylinositol 4,5-bisphosphate. Because this lipid is required for vesicle trafficking from lysosomes, we postulate that there is a defect in lysosomal enzyme trafficking in patients with Lowe syndrome that leads to increased extracellular lysosomal enzymes and might lead to tissue damage and contribute to the pathogenesis of the disease. We have measured seven lysosomal enzymes in the plasma of 15 patients with Lowe syndrome and 15 age-matched male controls. We find a 1.6- to 2.0-fold increase in all of the enzymes measured. When the data was analyzed by quintiles of activity for all of the enzymes, we found that 95% of values in the lowest quintile come from normal subjects whereas in the highest quintile 85% of the values are from patients with Lowe syndrome. The increased enzyme levels are not attributable to renal insufficiency because there was no difference in enzyme activity in the four patients with the highest creatinine levels compared with the six patients with the lowest creatinine values.

5-phosphatase | inositol signaling | phosphatidylinositol 4,5-bisphosphate | X-linked disorder | enzyme trafficking

Lowe syndrome is a disorder characterized by cognitive impairment with a discrete behavioral phenotype including temper tantrums, irritability, complex repetitive behaviors, and unusual mannerisms. Cataracts and severe visual impairment are present at birth, and most patients are blind eventually (1, 2). There is severe renal fanconi syndrome with progressive renal impairment. Growth failure is a prominent feature of the disorder, with most boys attaining a height of less than 5 feet. The disease is caused by mutations in an X-linked gene that encodes the OCRL (oculocerebrorenal:Lowe) protein (3–8). OCRL is an inositol polyphosphate 5-phosphatase that hydrolyzes all of the substrates of the enzymes in this family, including the inositol phosphates inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate and the inositol lipids phosphatidylinositol 4,5-bisphosphate [PtdIns(4, 5)P_2] and phosphatidylinositol 3,4,5-trisphosphate (9). A study using extracts from a renal proximal tubule cell line suggests that OCRL is mainly a lipid phosphatase (10). Thus, cultured cells from a patient accumulate PtdIns(4,5)P_2 despite the fact that these cells contain several other isozymes of inositol polyphosphate 5-phosphatase. This result implies that the different isozymes have discrete functions that are nonredundant. In renal proximal tubule cell lines from normal subjects, OCRL was shown to be localized to lysosomes by immunofluorescence (10). In the absence of OCRL, lysosomes may therefore accumulate PtdIns(4, 5)P_2. PtdIns(4, 5)P_2 is important for the formation of clathrin coated vesicles on lysosomes (11). Thus, in the presence of excess PtdIns(4, 5)P_2 in lysosomal membranes, there may be aberrant or increased membrane trafficking. We postulate that, in the case of Lowe syndrome, there may be mistrafficking of lysosomal enzymes, resulting in increased extracellular release of lysosomal enzymes. In the face of cells being continuously bathed in an excess of such hydrolytic enzymes, there may be tissue damage that contributes to the phenotype seen in Lowe syndrome. To evaluate this hypothesis, we have measured a panel of seven lysosomal enzymes in the plasma of boys with Lowe syndrome and age-matched male controls.

Methods

Lysosomal Enzyme Assays. Substrate 4-methylumbelliferyl-glycosides for each of the enzymes measured were purchased from Sigma. Assays were carried out at 37°C in 0.2 ml containing 75 mM sodium acetate (pH 4.75), substrate ranging from 0.0125 mM to 1.25 mM, and plasma. Each plasma sample was assayed for 15, 30, and 60 min. Reactions were stopped by the addition of 2 ml of 0.25 M glycine (pH 10.3). All assays were shown to be linear with time and plasma amount. Results are expressed as nanomoles of 4-methylumbelliferone formed/minute/milliliter of plasma. Plasma creatinine values were measured in the clinical laboratory of Barnes-Jewish Hospital.

Patients and Controls. Patients were recruited for the study with the assistance of the parental support group The Lowe Syndrome Association, Inc. Letters inviting participation were sent to the parents of boys aged 2 to 20 along with informed consent forms and a letter to the patients' physicians instructing them how to handle the blood samples. Blood (5 ml) was collected into EDTA and was centrifuged immediately, and the plasma was removed and frozen. Samples were shipped frozen to St. Louis and were stored at −80°C until thawed for assay. One sample was collected in our laboratory from a patient who lived nearby. Control blood samples were obtained from the outpatient hematology laboratory of St. Louis Children's Hospital from EDTA anticoagulated samples that were used for routine CBC. Control samples were taken to match the ages of the Lowe syndrome subjects from normal complete blood count samples. The control subjects had no chronic illness but were either normal or were seen for follow-up from fractures, other orthopedic problems, seizure disorder, or post-repair of a variety of congenital anomalies. Samples of both patients and controls were further identified only by number. The protocol and informed consent documents were approved by the Washington University Human Studies Committee.

Results

In preliminary studies, we measured lysosomal enzyme activity in fresh control plasma, in plasma frozen at −20°C for up to 4 months, and in plasma stored overnight at room temperature. There were no significant differences in the results obtained from these sources, and, thus, we instituted a study. We have measured seven lysosomal enzymes in 15 boys with Lowe

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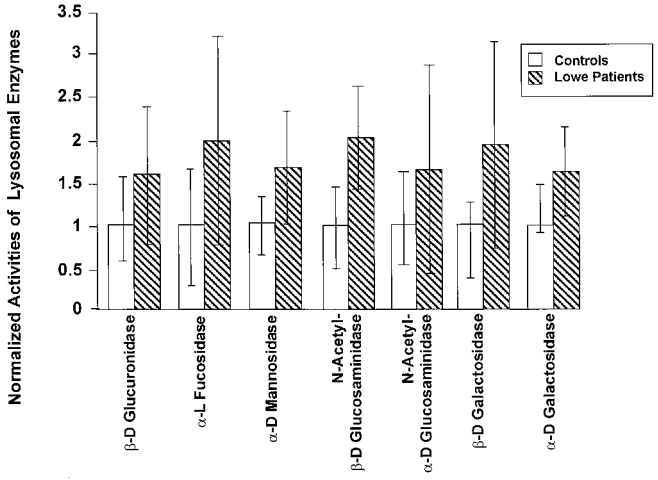

Fig. 1. Lysosomal enzyme activity in control vs. Lowe syndrome patients. The data are normalized so that the mean of the control samples for each enzyme is 1. The differences between control and Lowe syndrome values were evaluated by the Student's *t* test. The mean control values and significance of the differences were β-D-glucuronidase, 0.67 nmol/min/ml, $P = <0.05$; α-L-fucosidase, 3.71 nmol/min/ml, $P = <0.01$; α-D-mannosidase, 0.07 nmol/min/ml, $P = <0.01$; *N*-acetyl-β-D-glucosaminidase, 0.30 nmol/min/ml, $P = <0.001$; *N*-acetyl-α-D-glucosaminidase, 0.19 nmol/min/ml, $P = <0.1$; β-D-galactosidase, 0.0017 nmol/min/ml, $P = <0.01$; and α-D-galactosidase, 0.015 nmol/min/ml, $P = <0.01$.

syndrome and 15 age-matched male controls. All assays were done by the fluorometric measurement of released 4-methyl umbelliferone from the appropriate substrate. We measured β-D-glucuronidase, α-L-fucosidase, α-D-mannosidase, *N*-acetyl-β-D-glucosaminidase, *N*-acetyl-α-D-glucosaminidase, β-D-galactosidase, and α-D-galactosidase. The levels of all seven enzymes were increased in Lowe syndrome plasma from 1.6- to 2-fold, as shown in Fig. 1. All but one of the increases were statistically significant, as determined by Student's *t* test. The *P* values obtained ranged from $P < 0.1$ for *N*-acetyl-α-D-glucosaminidase to $P < 0.001$ for *N*-acetyl-β-D-glucosaminidase. The difference between Lowe syndrome patients and controls is more strikingly demonstrated when all of the assay values were pooled and ranked in quintiles from lowest to highest values, as shown in Fig. 2. In this case, the lowest quintile is almost all from controls with decreasing representation in each succeeding quintile. The converse is seen in Lowe syndrome, with few values in the lowest quintile and increasing numbers of values in the succeeding quintiles. We also measured plasma creatinine levels and plotted them vs. age to compare renal function in Lowe syndrome with that in controls. The normal increase in creatinine levels with age is defined by the equation creatinine = 0.35 + 0.03 × (age in years) as shown in Fig. 3. It is apparent that the slope of the increase is steeper in Lowe syndrome patients, indicating the slow progression of renal function impairment with age that has been reported previously (1). We considered the possibility that the high level of lysosomal enzymes in plasma from patients with Lowe syndrome might result from mild renal insufficiency. Thus, we compared the levels of enzyme activity in the six boys with the lowest creatinine values [$x = 0.47 +/- 0.05$ (SD)] with the four with the highest values [$x = 1.15 +/- 0.21$ (SD)]. As shown in Fig. 4, there was no difference between the enzyme levels that could be related to creatinine level. Of interest, it has been reported previously that plasma levels of the lysosomal enzyme acid phosphatase are markedly elevated in Lowe syndrome (1). In an effort to further elucidate the mechanism for increased lysosomal enzyme release in Lowe syndrome, we studied the uptake and release of β-glucuronidase in cultured renal tubular cell lines from a patient with Lowe syndrome compared with lines from control subjects. We could not detect any significant differences in either uptake or release of enzymes by these cell lines. This may reflect the fact that these cell lines do not mimic the physiological situation in the patients.

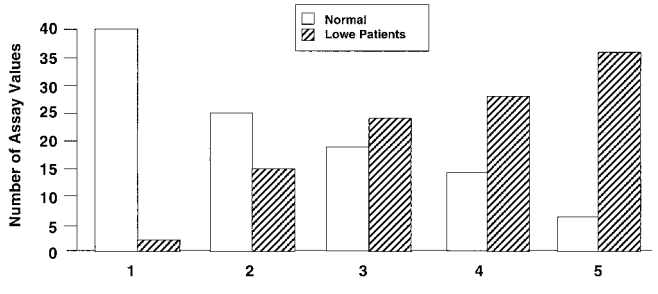

Fig. 2. Enzyme levels by quintiles Lowe syndrome vs. controls. Enzyme activities for each enzyme measured were ranked from lowest to highest and then were divided into quintiles. The number of values from control subjects vs. patients with Lowe syndrome in each quintile is plotted.

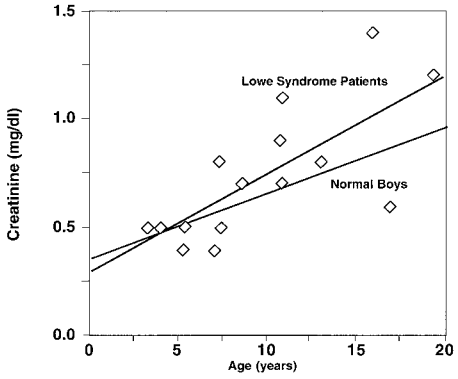

Fig. 3. Creatinine levels vs. age. The points plotted are those from patients with Lowe syndrome, and the line is from the least mean squares of these values. The line for controls is plotted from the equation creatinine = 0.35 + 0.03 × age in years.

Discussion

It is not clear how a defect in an enzyme of the inositol phosphate signaling pathway could lead to the complex phenotype of Lowe

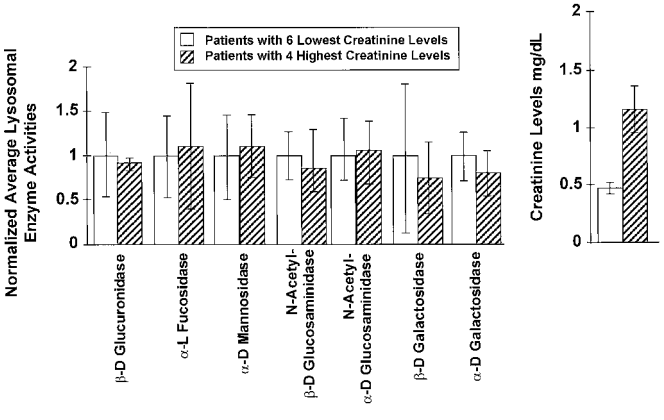

Fig. 4. Lysosomal enzyme activities vs. creatinine levels. Shown are plasma lysosomal enzyme activities of the Lowe syndrome patients with the six lowest creatinine levels vs. those with the four highest. The mean of the values of the lowest creatinines are normalized to 1.0. Error bars show standard deviations from the mean.

syndrome. The fact that the protein is widely expressed in essentially all tissues (12) and organs with the exception of blood cells while the defects are noted mainly in kidney, brain, and eye is also hard to understand. The OCRL gene has been deleted in mice by homologous recombination, but this experiment failed to yield insight into the disease because the deficient mice have no obvious phenotype (13). It would be of interest to study lysosomal trafficking in these mice or cell lines derived from them.

Our previous finding that the OCRL phosphatase is localized to lysosomes in renal proximal tubule cell lines and that the enzyme is primarily a PtdIns(4, 5)P_2 phosphatase in these cells suggested that there might be a defect in lysosomal enzyme trafficking in Lowe syndrome. The current results support this hypothesis, although there is little precedent for lysosomal hydrolases evoking tissue damage. There are suggestions that lysosomal hydrolases may play a role in cataract development, one of the phenotypes of Lowe syndrome (14). Muccolipidosis III is a disorder of lysosomal enzyme trafficking, and these patients have marked elevations of many lysosomal enzymes (15). The phenotype of this disorder is quite severe but does not include cataracts or renal insufficiency. An important mechanistic difference in this disorder is that lysosomal enzymes are secreted directly from the Golgi apparatus and not from the lysosomes, as we postulate happens in Lowe syndrome. Active hydrolytic enzymes secreted from lysosomes might be expected to cause more tissue damage than unactivated lysosomal proenzymes secreted from the Golgi (16). The most convincing method for proving that release of lysosomal enzymes accounts for pathogenesis in Lowe syndrome would be to treat patients with inhibitors of lysosomal hydrolases and demonstrate improvement in their clinical condition. To date, no such inhibitors are available to test this strategy. An alternative approach that we are currently pursuing is to stably transfect an immortalized lymphocyte cell line that does not express OCRL with a construct of OCRL in which expression is controlled by tetracycline. In this way, lysosomal enzyme trafficking can be evaluated in the presence and absence of expression of OCRL.

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