Increased levels of plasma lysosomal enzymes in patients with Lowe syndrome

Alexander J. Ungewickell and Philip W. Majerus*

Department of Internal Medicine, Division of Hematology, Washington University School of Medicine, St. Louis, MO 63110

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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.

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. Samples were stored overnight at room temperature.

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 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.

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

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syndrome and 15 age-matched male controls. All assays were done by the fluorometric measurement of released 4-methylumbelliferone 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 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, $P < 0.001$; N-acetyl-β-D-glucosaminidase, 0.30 nmol/min/ml, $P < 0.001$; N-acetyl-α-D-glucosaminidase, 0.19 nmol/min/ml, $P < 0.001$; β-D-galactosidase, 0.0017 nmol/min/ml, $P < 0.01$; and α-D-galactosidase, 0.015 nmol/min/ml, $P < 0.01$.

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, $P < 0.001$; N-acetyl-β-D-glucosaminidase, 0.30 nmol/min/ml, $P < 0.001$; N-acetyl-α-D-glucosaminidase, 0.19 nmol/min/ml, $P < 0.001$; β-D-galactosidase, 0.0017 nmol/min/ml, $P < 0.01$; and α-D-galactosidase, 0.015 nmol/min/ml, $P < 0.01$.

![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, $P < 0.001$; N-acetyl-β-D-glucosaminidase, 0.30 nmol/min/ml, $P < 0.001$; N-acetyl-α-D-glucosaminidase, 0.19 nmol/min/ml, $P < 0.001$; β-D-galactosidase, 0.0017 nmol/min/ml, $P < 0.01$; and α-D-galactosidase, 0.015 nmol/min/ml, $P < 0.01$.](image1)

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-D-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.

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

![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.](image2)

![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.](image3)

![Fig. 4. Lysosomal enzyme activities vs. creatinine levels. Shown are plasma lysosomal enzyme activities of the Lowe syndrome patients with 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.](image4)
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)P2 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). Mucolipidosis 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 mechanical 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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